Technical Bulletin 65 December 1929

FRANCIS J. RYAN
DEPARTMENT OF ZOOLOGY
COLUMBIA UNIVERSITY
NEW YORK 27, N. Y.

University of Minnesota
Agricultural Experiment Station

Mutation and Hybridization in Ustilago Zeae

PART I. MUTATION

E. C. Stakman, J. J. Christensen, C. J. Eide, and Bjorn Peturson

PART II. HYBRIDIZATION

J. J. Christensen
Division of Plant Pathology and Botany

UNIVERSITY FARM, ST. PAUL

FRANCIS J. RYAN
DEPARTMENT OF ZOOL
COLUMBIA UNIVERSITY
NEW YORK 27, N. Y.

University of Minnesota
Agricultural Experiment Station

Mutation and Hybridization in Ustilago Zeae

PART I. MUTATION

E. C. Stakman, J. J. Christensen, C. J. Eide, and Bjørn Peturson

PART II. HYBRIDIZATION

J. J. Christensen
Division of Plant Pathology and Botany

UNIVERSITY FARM, ST. PAUL

CONTENTS

Part I. Mutation

Page

Introduction 3

Previous work 5

Materials and methods 5

How mutants arise 7

Frequency of mutation 8

Mutant characters 14

- General cultural characters 14
  - General statement 14
  - Type of growth 16
  - Consistency of colonies 16
  - Direction of growth 18
  - Size of colonies 19
  - Elevation of colonies 23
  - Color of colonies 23
  - Surface characters of colonies 24
- Physiologic characters 25
  - General statement 25
  - Growth on standard bacteriologic media 27
  - Digestion of casein 30
  - Effect on sugars 31
  - Reduction of nitrates and iodine 31
  - Temperature relations 33
- Morphology of sporidia 35
- Pathogenicity and sex 36

Factors affecting mutation 43

- Nutrients 44
- Temperature 46
- Light 47

Degree of constancy of mutant characters 47

Does true mutation occur in *Ustilago zeae*? 53

Significance of mutation 61

Summary 63

Literature cited 64

Plates 67

Part II. Hybridization

Introduction 89

Are there "geographic strains"? 89

Terminology 91

Crosses made for study of f_1 lines 92

Characters of f_1 lines 93

- Sexual characters 93
- Further experiments with lines that caused infection singly 97
- Cultural characters 99
- Mutability 102
- Pathogenicity 102

Conclusions 105

Summary 106

Literature cited 107

Plates 109

FRANCIS J. RYAN
DEPARTMENT OF ZOOLOGY
COLUMBIA UNIVERSITY
NEW YORK 27 N. Y

MUTATION AND HYBRIDIZATION IN USTILAGO ZEAE[1]

Part 1. Mutation

E. C. Stakman, J. J. Christensen, C. J. Eide, and Bjorn Peturson[2]

INTRODUCTION

Ustilago zeae (Beckm.) Ung. comprises an indefinite number of unisexual lines that differ in so many physiologic characters that it seems justifiable to consider them physiologic forms (44, 45). It seems scarcely necessary to point out the importance of an intimate knowledge of physiologic specialization of plant pathogenes. A clear concept of the implications of the phenomenon is especially important in taxonomic work, in epidemiology studies, in establishing plant quarantines, and in breeding disease-resistant varieties of crop plants (8, 43). But it is important not only to know the number, pathogenic capabilities, and geographic distribution of forms now in existence but also to learn how commonly new forms are coming into being. The more precisely we can know what to expect, the more intelligently we can act.

We know that corn smut is a destructive disease that can be controlled adequately only by using resistant varieties, and we know that such varieties can be produced (26, 30). But we know also that there are many physiologic forms of *U. zeae*, the pathogene causing most of the smut in the corn belt of the United States, and that selfed lines of corn may be resistant to some combinations of forms and susceptible to others (15). Assuming that some lines are resistant to all existing smut forms, it would be highly desirable to know whether new forms might arise to which they would be susceptible. This, then, raises the question as to the genetic stability of physiologic forms.

Contrary to the older belief that differences between physiologic forms of many fungi are only phenotypic, there is abundant evidence that they are genotypic. The idea that forms change readily by a process of education or adaptation has been disproved to a considerable extent (43). This does not mean that new forms may not appear but that physiologic forms of fungi probably are not unique genetically. Their physiologic and parasitic characters are due to the interaction of genetic and environmental factors, as are the characters of higher

[1] This manuscript was submitted for publication in October, 1929.

[2] The writers are greatly indebted to numerous pathologists and agronomists in the United States and to Dr. Bela Husz of Hungary, Dr. E. Pantanelli of Italy, Dr. Louis M. Unamuno Yrigoyen of Spain, and Dr. J. Dufrenoy of France, for collections of corn smut. They are also indebted to Dr. W. F. Hanna for certain monosporidial isolations and to R. H. Bamberg and C. S. Holton for assistance in making monosporidial isolations and inoculations.

plants. Consequently the laws of inheritance apply in both cases. There seems to be no valid reason, then, for supposing that new physiologic forms originate otherwise than by hybridization and mutation. That they probably arise in both these ways in *U. zeae* has been shown by Christensen and Stakman (15); Stakman and Christensen (44); Stakman, Christensen, and Hanna (45); and Hanna (24). But how often and under what circumstances they arise and what their pathogenic capabilities are after they have arisen is only imperfectly known. The writers have attempted an extensive study of physiologic specialization in *U. zeae,* because of its practical importance and because this species is very suitable for a study of the nature and origin of physiologic forms.

The mature chlamydospores of *U. zeae* are diploid. On germination they normally produce a promycelium in which reduction division apparently occurs (24). The sporidia typically are uninucleate, altho they sometimes contain more than one nucleus, and the progeny of a single sporidium should constitute a clonal line, as it buds in a yeast-like manner and thus multiplies indefinitely by vegetative propagation. These haploid or gametic clonal lines grow readily in artificial culture, and single sporidia can be isolated easily; hence studies of mutation can be made under unusually favorable circumstances. Furthermore, *U. zeae* usually is heterothallic, sexual fusions being prerequisite to normal infection in most cases (19, 24, 44). There is a relatively short dikaryophase, followed by nuclear fusion and formation of the diploid chlamydospores. These chlamydospores usually are produced about two weeks after the plants are inoculated, and they germinate readily; consequently only a short time is required for hybridization studies.

But there are difficulties in determining the pathogenicity of the new physiologic forms that arise by mutation and hybridization. Most monosporidial lines alone cannot cause normal infection. They represent the saprogenic phase in the life history of the pathogene. Moreover, the dikaryophytes and diplonts apparently cannot be produced on artificial culture media, but only in the living host. Therefore it is necessary to determine the pathogenicity of combinations of forms, as that of the individuals alone cannot be determined. This condition in the smuts is somewhat unique, being quite different from that in most other pathogenic fungi, in which individual monosporous lines can cause infection.[3]

It was the object of the present investigation to study the genetic stability of *U. zeae* to obtain facts of value in the solution of the corn

[3] While this manuscript was being prepared, evidence was obtained by one of the authors (Christensen) that some monospordial lines could cause infection when inoculated singly into corn plants. The results are presented in Part II.

smut problem, and particularly to learn as much as possible about the general problem of the origin, nature, and importance of physiologic forms of pathogenic fungi. About 200 monosporidial lines of *U. zeae* have been studied more or less intensively, particularly with reference to mutation and hybridization.

PREVIOUS WORK

Kniep (31, 32) and Zillig (47), in their investigations of *Ustilago violacea* (Pers.) Fuck., called attention to the gametic nature of sporidia and thus laid the foundation for studies of hybridization and mutation in the Ustilaginales.

Bauch (1), in 1925, was the first to record mutation in the smut fungi. He observed the phenomenon in *Ustilago bromivora* (Tul.) F. de W. Christensen and Stakman (15), Stakman, Christensen, and Hanna (45), and Hanna (24) demonstrated that mutation is very common in *U. zeae*. Stakman, Christensen, and Hanna stated that a very large number of mutants might arise from monosporidial lines, which they consider haploid clones. They pointed out that the mutants might differ consistently from their parents and from each other in one or more of the following respects: morphology, cultural characters, and apparently sometimes in sex and potential pathogenicity. According to these authors, the frequency of mutation can be influenced by certain environmental conditions, altho suitable media often are necessary to bring out the differences between mutants.

Part I of this bulletin records the data on mutation obtained by the writers during the past two years, and Part II gives the results of studies on hybridization.

MATERIALS AND METHODS

More than 200 monosporidial lines of *U. zeae* were isolated and observed to some extent in the study of mutation. The method of isolating sporidia was that described by Hanna (23). In some cases the individual sporidia were isolated directly from the promycelium, in others shortly after the sporidia had become detached, and in still others from cultures of smut lines that had been grown for many months, or, in some cases, years.

The collections from which these monosporidial lines were derived were obtained from many different localities in the United States, from Canada, and from Hungary, Italy, France, and Spain. When cultures were made from collections of chlamydospores, the spores were soaked for twenty-four hours or longer in a 1 per cent copper sulfate solution and then used either for pouring dilution plates or for making streak inoculations on agar. The individual sporidia were then isolated from the cultures.

Monosporidial lines are designated by the locality from which they were obtained. If several distinct lines were isolated from a promycelium, locality, or collection, each was given a letter of the alphabet. Thus Minn. A, Minn. B, Minn. C, and Minn. D were isolated from the same promycelium of a chlamydospore from a Minnesota collection. The chlamydospores of an Italian collection received from Dr. Pantanelli were plated out by the dilution method, and there were several types of resulting colonies. Transfers were made from them, and it was evident that there were several distinct cultural types. These lines were designated Italy A, Italy B, Italy C, Italy D, etc. Again, Dr. R. J. Garber sent three packets of chlamydospores from West Virginia, labeled "leaf strain," "basal strain," and "ear strain," indicating the region of the plant on which the galls had been produced. The lines isolated from these collections were named W. Va. A, W. Va. B, W. Va. C, respectively. All three lines showed a pronounced tendency to mutate, but mutations were especially numerous in line A. Nine mutants appeared within a short time and these were given numbers, thus: W. Va. A1, W. Va. A2, and so on to W. Va. A9. W. Va. A8 seemed a particularly promising line on which to make observations, as it soon produced eight distinct mutants. These in turn were numbered as follows: W. Va. A8-1 and so on to W. Va. A8-8. W. Va. A8-5, for example, indicates the fifth mutant from A8, which in turn is the eighth mutant from the original culture, W. Va. A. When the identity of a line is perfectly clear, as in tables and discussions of a series of mutants from one line, the locality designation sometimes is omitted for brevity. Thus, when it is clear that W. Va. A8-5 is meant, it is referred to simply as A8-5. When a number precedes the symbol for a given line, it shows how many times that line had been transferred in culture. For example, 10 W. Va. A8-2 would mean that W. Va. A8-2 had been transferred 10 successive times, and therefore had been in culture for a long period. This number is omitted except in certain cases in which it seems desirable to indicate the number of transfers that had been made.

Many different kinds of nutrient media were used during the course of the work. For stock cultures ordinary 1 per cent potato-dextrose agar generally was used. "Difco" agars were used for more careful work. The organism grows well on ordinary potato-dextrose agar, on potato-dextrose agar plus half strength Shive's solution, on potato-dextrose-maltose agar, and on several other media. While several different media were used, some of which cannot be standardized accurately, the media on which smut lines were grown for comparison always were made up in one batch and equal amounts were poured into each flask to be inoculated.

Stock cultures were grown in test tubes but other cultures were grown in Erlenmeyer flasks of about 250 cc. capacity, where differences in cultural characters were readily observed and mutants could be seen to best advantage.

HOW MUTANTS ARISE

Mutating colonies are clearly shown in Plate I. It will be noted that most of the mutants appear as wedge- or fan-shaped sectors in the colony; but they may appear also as irregular patches at various places on the surface of the colony, as described by Brown for Fusarium (8). These patches can be seen clearly in Plate I, B. A large percentage of the sectors are likely to develop near the edge of the parent colony, while the patches are most likely to appear nearer the center. Sometimes the margin of a colony may be almost entirely occupied by sectors of different sizes, and, in a few cases observed, the surface of a colony was covered almost completely with "patch mutants." The sectors vary greatly in shape, size, surface characters, consistency, sharpness of outline, and color, as will be noted from Plate I and Figure 3. By far the largest number of mutants observed by the writers appeared as rather sharply-defined sectors, and relatively few as patches.

Clearly defined sectors not infrequently appear within sectors. For example, in a colony of W. Va. A8-4-3 there were 6 distinct sectors near the margin, and a number of patches near the center of the colony. The colony itself was dirty yellow in color and the surface was coarsely reticulated, almost morel-like in appearance. One of the sectors was large, fan-shaped, smooth, and pale yellow; three of them were small, wedge-shaped, smooth, and bright yellow; the fifth was irregularly rectangular, smooth, except for tangential striae, and fuliginous in color. Within the last sector, and extending to the margin, there was a sharply-defined, black, wedge-shaped sector. The patches near the center were dull black.

It is evident that there would be no difficulty in detecting sectors like those shown in Plate I. But it is more difficult to see the patch mutants, especially when they are small and poorly developed. Under such circumstances it is often extremely difficult to make transfers from them, particularly if they are tough or viscous. One cannot always be sure of recovering even the parental form, as it seems certain that there may be many mutant primordia that are not observable at all. For this reason the writers sometimes restricted their efforts largely to the isolation of lines from the better-defined sectors in colonies that appeared relatively homogeneous except for the presence of the sectors, altho an attempt was made to take the mutants as they came.

Nevertheless, many probably were neglected, but there were several thousands more than could have been studied in any case, so the neglect did not seem serious.

FREQUENCY OF MUTATION

The 200 monosporidial lines isolated were distinctly and consistently different from each other in cultural characters, and, in our opinion, may properly be considered physiologic forms, altho they usually are referred to as lines in this paper. *U. zeae* evidently comprises quite as many distinct lines as does corn. And this is not surprising when one observes how readily some lines of the fungus mutate.

Most of the monosporidial lines studied have matured to a greater or less extent; altho some mutated more frequently than others. The 200 lines have given rise to thousands of mutants during a period of two years. Stability is the exception to the rule—even those lines that appear to be stable for a long time may suddenly mutate abundantly. This was true of Canada A and Texas A, which had been grown in culture about four years without mutating. Suddenly they

TABLE 1

NUMBER OF MUTANTS PRODUCED BY MINN. A AND TWENTY OF ITS MUTANT LINES WHEN GROWN AT ROOM TEMPERATURE ON TWO DIFFERENT KINDS OF AGAR MEDIA, IN TRIPLICATE FLASKS OF EACH

Lines	Medium and number of mutants*		
	Potato dextrose + 1 per cent malt	Potato dextrose + 1 per cent malt + 2 per cent sucrose	Totals
Minn. A	6	9	15
A1	0	2	2
A1-1	0	0	0
A2	8	4	12
A3	8	7	15
A3-1	5	7	12
A4	8	0	8
A4-2	5†	5	10
A4-3	9†	6	15
A5	5	7	12
A5-1	8	6	14
A6	2	3	5
A7	0	0	0
A8	0	0	0
A9	0	1	1
A10	0	0	0
A11	0	0	0
A11-1	4†	6	10
A12	8	1	9
A13	..	0	0
A14	..	17	17
	76	81	157

* The potato-dextrose agar was 4 per cent "Difco"; the malt ..as Merck's powdered malt extract.

† Duplicate flasks.

began to mutate and are continuing to do so. Mutants may in turn mutate, also in varying degrees. (See Fig. 3.)

The differences in the tendency of various mutant lines to mutate are shown in Tables I, II, and III.

Of the 21 lines listed in Table I, all but 6 mutated, 17 being the largest number of mutants produced by any one line in the 6 flasks. In all, there were 123 flasks, one colony in each, and 157 mutants appeared in them. Of these, 69 were distinct, the others apparently being duplicates.

Table II shows clearly the differences in the tendency of different lines to mutate. In 6 colonies of W. Va. A8-4-3 there were 38 distinct mutants, an average of more than 6 in each colony, while there were only 2 in 5 colonies of A8-4, the immediate parent. There also were 38 mutants in 6 colonies of A8-5-3. On the other hand, there were none in 4 flasks each of A8-4-2 and A8-4-5. In a total of 64 colonies in this experiment, 134 mutants appeared, an average of more than 2 a colony, and 2 lines of the 12 contributed more than half of this number. But perhaps the most conspicuous example of differences in lines with respect to mutation is the fact that, of the 162 distinct mutants derived from W. Va. A8, one line, A8-5, gave rise to 112 in 5 cultural generations, while A8-7, A8-8, A8-9, and A8-10 gave rise to none at all.

TABLE II

NUMBER OF MUTANTS THAT APPEARED IN CERTAIN MUTANT LINES OF *Ustilago Zeae* DERIVED FROM W. VA. A8, GROWN ON THREE DIFFERENT NUTRIENT AGAR MEDIA IN DUPLICATE FLASKS FOR EACH MEDIUM

Lines	Medium and number of mutants			
	Potato dextrose	Potato dextrose plus half strength Shives' solution	Litmus lactose	Totals
W.Va A8-4	2	1	0*	3
A8-4-1	4	0	4*	8
A8-4-2	0	0	..†	0
A8-4-3	12	25	1	38
A8-4-4	2	..	1*	3
A8-4-5	0	0	..†	0
A8-5	1	9	2*	12
A8-5-1	5	2	3	10
A8-5-2	2	0	2	4
A8-5-3	9	20	9	38
A8-5-4	2	1	2	5
A8-5-5	5	2	7	14
	44	59	31	134

* One flask only.
† Both flasks discarded.

Two sporidia were isolated from a single promycelium and observations made to determine the relative frequency of mutation in the two resulting monosporidial lines, Minn. A and Minn. D, grown on 6 different culture media. The results are given in Table III. It will

be noted that Minn. A produced 33 mutants, some on all media except one. Only 5 indistinct sectors appeared in colonies of Minn. D, on the other hand. This indicates that there probably was a segregation of factors that either directly or indirectly govern mutability. It had been found previously that the two lines were of opposite sex;[4] therefore segregation of sex factors certainly had taken place.

TABLE III

NUMBER OF MUTANTS THAT APPEARED IN CULTURES OF TWO MONOSPORIDIAL LINES OF *Ustilago Zeae* DERIVED FROM SPORIDIA OBTAINED FROM THE SAME PROMYCELIUM

Medium*	No. flasks	Lines of *U. zeae* and number of mutants	
		Minn. A	Minn. D
4.5% Potato dextrose	2	4	0
3.5% do	2	6	0
2 % do plus 2% cornmeal	3	8	0
4 % do plus 0.5% peptone	3	6	5†
4 % do plus 2% sucrose	3	8	0
Half strength Shive's solution plus 1.2% agar plus 3% dextrose	3	1	0
Half strength Shive's solution plus 1.2% agar plus 3% dextrose plus 3% lactose	3	0	0
Total	19	33	5

* "Difco" potato dextrose and cornmeal agars were used.

† Sectors in Minn. D not sharp.

It seems evident from Tables I, II, and III not only that there are wide differences in the mutability of monosporidial lines but that mutation occurs with surprising frequency in some. To study all of the thousands of mutants that appeared was obviously impossible without enlisting the services of a small army of investigators. It was decided, therefore, to select two lines for more intensive study.

Detailed observations were made on two monosporidial lines of opposite sex, W. Va. A8 and Minn. A. The latter produced 70 distinct mutants and the former, 162 by the fall of 1928. Since that time many more have appeared, but they are not recorded here. Figures 1 and 2 represent graphically the sequence of origin of the first 162 and 70 mutants of these two lines, respectively.

As the largest number of mutants came from W. Va. A8, a brief history of this line is given. The original chlamydospore material was obtained from Dr. R. J. Garber, of the University of West Virginia. In the early spring of 1927 dilution plates were poured. On May 3 transfers were made to three flasks of potato-dextrose agar, and nine sectors appeared in the three colonies. These sectors were considered probable mutants, despite the fact that monosporidial isolations had not yet been made. It is possible that they were segregates, but this seems improbable because no colonies resembling them had appeared in the

[4] Dr. W. F. Hanna made the original sex determinations.

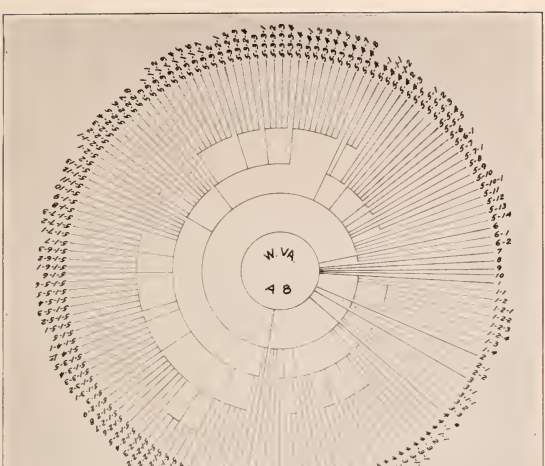


Fig. 1. Diagram showing the origin of 162 mutants from one monosporidial line of *U. zeae*, W. Va. A8.

original plates. Furthermore, the sectors appeared near the periphery of colonies about 35 mm. in diameter, and it seems likely that the colonies would have appeared heterogeneous while still fairly young, had several segregates been growing together. In fact, flasks were inoculated at various times with two or more lines of distinctly different appearance, and, altho it could be seen clearly that the colonies comprised several lines, these lines did not arrange themselves into sectors. It seems probable, therefore, that A8 was a mutant, altho it is not certain. The parent colony was light buff to tan in color, with a pinkish tinge near the center. The surface was slightly coral-like near the center and dull, china-like elsewhere. It was beautifully marked with very fine concentric lines. The sector was decidedly olivaceous, smooth except for fine concentric lines like those on the parent colony, and bordered with a feathery to powdery fringe. A transfer was made from this sector and several successive transfers were made subsequently. In January, 1928, a single sporidium was isolated, and the resulting line was then used for the study of mutation.

The monosporidial line of W. Va. A8 began to mutate as soon as colonies resulting from transfers became available for study. By

March 8, mutants A8-1, A8-2, etc., had appeared. They were compared under uniform conditions and were decidedly different in appearance on several kinds of culture media. All of these mutants in turn mutated except A8-7, A8-8, A8-9, and A8-10. By the fall of 1928 a total of 220 mutants had been isolated, and it was decided to discontinue selecting and culturing new ones which were appearing, as the process seemed to be going on ad infinitum.

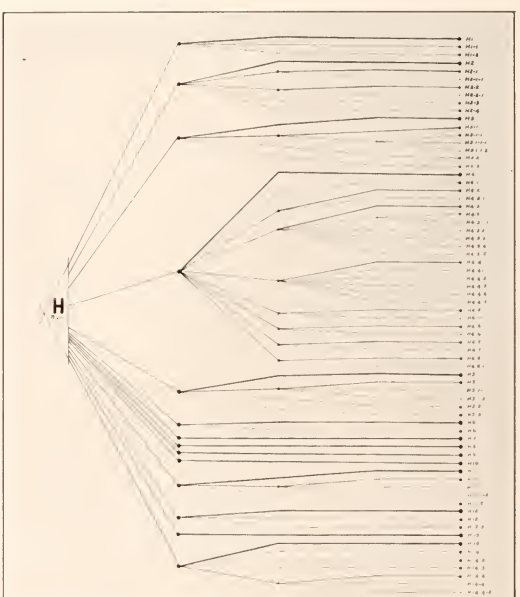


Fig. 2. Graphic representation of the origin of 70 mutants from one monosporidial line of *U. zeae*, Minn. A (designated in diagram by H).

The 220 mutants were transferred on November 24 to agar slants in order to obtain cultures of the same age for inoculating flasks for comparison of cultural characters. On December 1, each mutant line was transferred to triplicate 250 cc. Erlenmeyer flasks, each containing 35 cc. of nutrient agar made up of potato extract, 1.8 per cent agar, 1 per cent dextrose, 1 per cent sucrose, and 1 per cent Trommer's malt

extract. It is realized, of course, that the composition of a medium made up with potato extract as a base might vary greatly when made up at different times, but the objection of variability would not apply to a single comparative test such as was made in this case. The agar was made up in one batch, 35 cc. poured into each flask, and all of the 660 flasks sterilized at the same time in a large steam-pressure sterilizer.

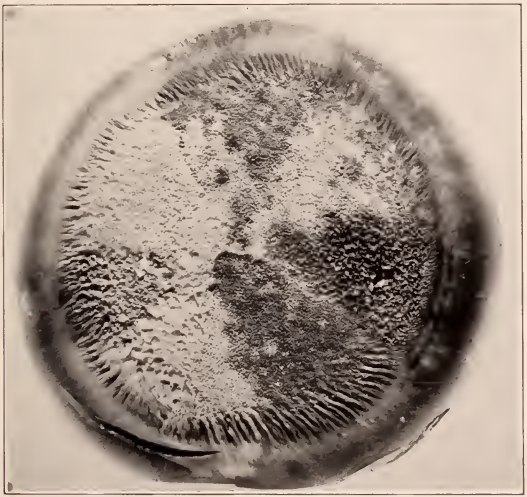


Fig. 3. Minn. A3-1-1-2-1-1, which originated through six successive mutations, still producing mutants.

At the end of four weeks notes were taken on the cultures. It appeared that 38 lines were duplicates, 4 triplicates, and 4 quadruplicates, leaving 162 that were decidedly distinct from the parent and each other, with the possible exception of one that resembled the parent somewhat and one that apparently had lost the ability to grow. Of the 162 distinct lines, 112 were derivatives of A8-5, showing again the differential tendency of lines to mutate. The 58 discarded lines may have been different in characters other than gross cultural characters, or they might have been distinct in appearance had they been grown on other culture media, so it is unsafe to conclude that they actually were identical with some of the other lines. However, the amount

of work required to settle the question seemed disproportionate to the probable value of the results, and the task was not undertaken. It is known, of course, that apparently identical mutants may arise independently and that there may be mutations to the parental type; consequently it would not be surprising to find almost identical lines among a large number of mutants.

Minn. A also mutated very frequently. It is noteworthy that the first 70 mutants isolated in succession from this line were distinctly different from the parent and from each other.

Some of the mutants were relatively stable in culture, while others were very unstable. (See Tables I, II, and III.) No mutations have been observed in Minn. A9 and Minn. A10, for example, altho they have been grown for 21 months on many different kinds of media. It is not unlikely, however, that they may begin to mutate at any time. Minn. A13 had been grown in culture for more than a year and had been transferred at least a dozen times, without any indication of mutation until the winter of 1928-29, when it suddenly began to mutate and has continued to do so through several successive cultural generations.

There were similar differences in the mutability of mutant lines of W. Va. A8. For example, A8-1 gave rise directly to four mutants which in turn gave rise to 13, many of which again mutated. In one case 15 mutants appeared in a single colony of W. Va. A8-4-3. Of the 162 distinct lines, 112 were derivatives of A8-5. On the other hand, A8-7, A8-8, A8-9, and A8-10 have produced no mutants whatever.

MUTANT CHARACTERS

Mutants may differ from their parents in several respects. They may differ decidedly in cultural characters, including rate of growth, direction of growth, general appearance of the colonies, and pigmentation. There is evidence that morphological changes also may occur. Parent colonies may consist of sporidia, and the mutants almost entirely of mycelium or vice versa. The mutants may differ from their parents also in general physiologic characters, in their tendency to mutate, in sexual characters, and in pathogenicity.

General Cultural Characters

General statement.—Mutants may differ from their parents in one or more of the following general cultural characters: rate of growth, type of growth and consistency of the colony, surface characters, and color.

An idea of the differences in general cultural characters of mutants can be gained from Tables IV and V, in which are listed some of the characteristics of mutants grown on potato-dextrose-maltose agar and

on 2 per cent potato-dextrose agar plus a trace of methyl orange. It is perfectly clear from Tables IV and V and from Plates II, III, and IV that there may be very wide differences in the general appearance of colonies of mutants which originated from the same monosporidial line. Numerous comparisons similar to those listed were made with these same mutants and with many others. As a matter of fact, the principal cultural characters of the 70 mutants in the Minn. A series and the 162 derived from W. Va. A8 have been carefully recorded, but it seems scarcely worth while to give them all in detail.

The following notes taken on colonies in triplicate flasks of potato-dextrose agar may give an idea of the difference in appearance of mutants from the same parent.

W. Va. A8-4: Colonies 40 mm. in diameter, roughly convoluted, orange-buff in color; mid-size radial ridges, about 5 mm. long, extending in clockwise direction near the edge of the colony; delicate opaque margin, about 6 mm. wide

W. Va. A8-4-1: Colonies 43 mm. in diameter, less convoluted than those of A8-4, with a tendency to form fine ridges radiating from the center and ending in radial ridges as above, but extending in a decidedly counter-clockwise direction; margin about as in A8-4; color approximately the same as in A8-4.

W. Va. A8-4-2: Colonies 43 mm. in diameter; surafce convoluted into low folds; radial ridges somewhat as in previous two lines, and extending in slightly clockwise direction; central area of colony grayish-buff in color, and margin pearl-gray.

W. Va., A8-4-3: Colonies 50 mm. in diameter; grayish to mauve in color; surface coral-like; colony very thin; margin pale ivory in color.

W. Va. A8-4-4: Colonies 40 mm. in diameter; coarsely convoluted as in A8-4; center orange-brown in color and remainder deep-buff orange; radial ridges similar to those of A8-4 but much finer and straight or very slightly clockwise.

W. Va. A8-4-5: Colonies 40 mm. in diameter, coarsely convoluted; very coarse and distinct radial convolutions and deep marginal ridges; colony deep orange throughout except on the marginal ridges which are somewhat paler in color than the rest of the colony.

The salient cultural characters of 8 mutant lines of W. Va. A8 are given in Table IV and are shown in Plate IV. The different lines had been grown in culture from 10 to 14 months, on many kinds of media, and under various environmental conditions. Late in July, 1929, all were transferred to flasks of potato-dextrose-malt agar for temperature relation studies. Notes on cultural characters of duplicate flasks incubated at 26° C. were taken on September 1, 1929. The duplicate colonies of the same line were as nearly identical in appearance as could have been expected. But the appearance of those of different lines differed so greatly that several people, who scarcely knew the meaning of the word fungus, could readily sort the lines from a random arrangement into the proper groups.

Descriptions and uncolored photographs are quite inadequate to convey a clear idea of the remarkably nice differences in appearance

of cultures of different lines. To paint a faithful word picture of the colonies is virtually impossible; and photographs do not show the sometimes decided and sometimes slight but extraordinarily beautiful differences in color. In descriptions of cultural characters differences may appear to have been drawn too finely to be real, but the astonishing fact is that they actually are so very real and so entirely characteristic of certain lines of the fungus.

It will be seen from Table IV and Plate IV that cultures of the lines listed differed from each other considerably in size of colony, color, luster, surface, and margin. In fact, so different are the cultural characters of different mutant lines grown under uniform conditions that many lines would be suspected of belonging to different species.

Type of growth.—Some lines may produce mycelium and others sporidia almost exclusively. A mycelial colony may produce aerial sporidia, and hyphae may be present to a certain extent in sporidial colonies, depending on the culture medium and other conditions. In other cases, however, the colonies consist almost exclusively of mycelium or sporidia. For example, W. Va. B was wholly mycelial whereas its mutant, W. Va. B5, was wholly sporidial. Sporidial colonies often produce mycelial mutants which usually grow more rapidly than the sporidial parent and can be more easily selected and propagated. As a matter of fact, it seems likely that the difficulty of maintaining the sporidial type in stock cultures is due largely to the fact that mycelial mutants develop abundantly, grow rapidly, and therefore are likely to be transferred instead of the sporidial parent. Some sporidial lines, however, can be propagated and maintained for a long time in culture if sufficient care is exercised in making transfers. Texas A, a sporidial type, for example, has been kept in culture for at least four years, and several other lines have been maintained for more than a year, but they have given rise repeatedly to mycelial mutants. Both mycelial and sporidial lines usually give rise to mycelial mutants, but not necessarily so. As indicated, some mycelial lines have produced sporidial mutants also.

In colonies of mycelial lines the hyphae may be coarsely tufted, finely tufted, or even silky. There appear to be all gradations in the size of hyphae as well as in the manner in which they are aggregated into mats of woolly, felty, cottony, or silky appearance and texture.

Consistency of colonies.—As would be expected from the fact that there is so great a range in the type of growth, there is the greatest range in the texture and consistency also, as they depend to a considerable extent on the type of growth.

The consistency of colonies on agar media may be slimy, butyrous, viscid, brittle, powdery, membranous, or coriaceous. And sectors of any one of these consistencies may arise in colonies of any other type

TABLE IV

Cultural Characters of Eight Lines of W. Va. A8 Derivatives Growing in Duplicate Flasks of Potato-Dextrose Agar

Line	Diam. in mm.	Color	Elevation	Luster	Surface and topography	Edge
A8-5-3-3-2	50	White; faint purple brown near center	Convex	Cretaceous	Verrucose	Slightly fimbriate
A8-5-4	43	Orange brown; margin faint orange, covered with white powder	Broadly umbonate	Dull in center; marginal band powdery	Somewhat contoured; marginal band with small radiating ridges	Erose to lobate
A8-3-1	56	Gray drab on central knob; surrounded successively by olive drab, gray drab, and dirty yellowish-white zones	Convex-umbonate	Dull to cretaceous	Central area rugulose; surrounded by coarsely rugose zone, and broad marginal zone almost flat; radial folds extending 1 centimeter from central area but not reaching margin	Fimbriate
A8-1-1	18	Pinkish buff	Pulvinate	Dull waxy	Rugose to rugulose	Finely lobate to auriculate
A8-5-7-1	50	Gray brown in central zone; surrounded by narrow dark brown band, then buff brown and margin brownish gray	Flat	Dull	Zonate; smooth, finely felty	Somewhat plumose
A8-5-2-1	40	Whitish brown to smoke gray	Raised to convex	Cretaceous	Rugose; irregular radial furrows near margin and tendency for folds to crack irregularly	Somewhat lobate
A8-5-5	55	Orange buff	Convex	Dull	Contoured; regular radial furrows in marginal zone	Entire
A8-5-3-2-5	45	Purple brown, with white powder in central and marginal zones	Convex	Dull to cretaceous	Coarsely verrucose; closely branched radial ridges in marginal zone	Undulate

of consistency. For example, the colonies of a monosporidial line of Italy C were dark purple in color and felt-like or coriaceous in consistency on potato-dextrose agar. Two large sectors appeared in one colony, one of which was cinnamon brown with a tinge of purple in color and rather brittle in consistency, while the other, a very rapid grower, was Jews-ear fungus-like in appearance and butyrous in consistency. On the other hand, the consistency of colonies of W. Va. A8-3 was butyrous, but that of A8-3-1, which appeared as a large, fan-shaped sector, was decidedly coriaceous. Again, the consistency of A8-4 was brittle, while that of 4 sectors ranged from membranous to coriaceous.

There are all degrees of viscosity. Transfers are easily made from some colonies, especially the butyrous type. Inoculum can be obtained by dipping a loop of small gage wire into the colony. Some colonies are so viscid, however, that it is only with great difficulty that inoculum can be obtained, even with a stiff wire. Colonies may be only slightly viscous while others may be doughy or even gummy in consistency. Still others may be so brittle or powdery that it is necessary to moisten the inoculating needle before sufficient inoculum will adhere to it. Some colonies are so membranous that it is necessary to cut out small squares to avoid lifting the entire colony with the inoculating needle. It was necessary to make a small, sharp-edged spatula to cut small pieces of inoculum out of some of the colonies that were tough, felty, or almost leathery. Colonies also may be cottony, silky, or fluffy. And all of these types can be found in mutants derived from one or two monosporidial lines.

Direction of growth.—The direction of growth often is subject to mutation. The mycelium of some mutants has a tendency to grow in a clockwise direction, that of others in a counter-clockwise direction, and that of still others in a strictly radial direction. Mutants characterized by any one of the three directions of growth may be obtained from the same line, sometimes from the same colony. (See Table V.) The difference in direction of growth is illustrated in a series of mutants which arose in triplicate flasks of W. Va. A8-4. The direction of growth is indicated in the following summary:

A8-4: Mid-size ridges about 5 mm. long near the margin of colony; direction decidedly clockwise.

A8-4-1: Fine radial ridges extending to within about 10 mm. of edge, then an area of ridges as above, but direction decidedly counter-clockwise.

A8-4-2: Fine radial ridges; direction slightly clockwise.

A8-4-3: No ridges.

A8-4-4: Ridges as in A8-4, but finer and straight or very slightly clockwise.

A8-4-5: Very large ridges near margin; direction straight.

Mutation in direction of growth is illustrated also by Italy A. The parent colonies were lavender to purple in color, and smooth except

for rather fine radial ridges extending from the center about half way to the margin. There was no appreciable turning of the ridges in either direction. A1 and A2 arose as sectors. A1 was quite similar to the parent except that the radial ridges or folds were much larger, extended farther toward the margin, and turned in a decidedly clockwise direction. In A2 the color was grayish buff and the whole surface was covered with coarse ridges that united to form a beautiful reticulum, the colony resembling the surface of a small morel.

Microscopic examination was made of A and A1 and it was found that the hyphae of A grew straight, while those of A1 curled, explaining the clockwise growth of the ridges.

Direction of growth is somewhat variable but there is a general tendency for it to be fairly constant under one set of conditions, altho not necessarily so under different conditions. The counter-clockwise direction of growth is illustrated in Plate III.

Size of colonies.—Mutants may grow more rapidly or more slowly than their parents. Many observations and tests were made to ascertain the difference in rapidity and extent of growth of mutants

TABLE V

SIZE, TYPE OF GROWTH, DIRECTION OF GROWTH, AND NUMBER OF SECTORS IN COLONIES OF 16 MUTANT DERIVATIVES FROM MINN. A GROWN ON 2 PER CENT POTATO-DEXTROSE AGAR PLUS A TRACE OF METHYL ORANGE

Mutants	Diameter of colony in mm.	Direction of growth*	Type of growth	Aerial† mycelium	Number of sectors
Minn. A2-2-1	33	cc+	Mycelial	0	6
A2-4	31	c±	do	++	10
A3-2	32	cc±	do	0	1
A3-1-2	36	cc++	do	+++	0
A4-2-1	30	0	do	0	1
A4-3-5	23	0	do	0	7
A4-4-3	28	cc++	do	0	9
A4-4-4	31	0	Sporidial	0	0
A4-6-1	29	cc+++	M celial	++	2
A5-2	38	0	Sporidial	0	0
A12-1	38	cc+	Mycelial	0	3
A13	47	0	Sporidial	0	0
A14	28	0	Semi-sporidial	0	25
A14-2	38	0	do	±	13
A14 3	28	0	do	0	19
A14-4-2	32	c+	Mycelial	++++	11
					107

* cc = counter-clockwise growth; c = clockwise growth; + = degree of turning.

† 0 = no aerial mycelium; + = aerial mycelium present, the amount indicated by number of + signs; ± = slight amount.

as compared with that of their parents and other mutants. Observations usually were made in triplicate flasks for each medium, altho sometimes duplicates were used. The diameter of the colonies was measured in two directions at right angles to each other, and when the colony was not circular the results were averaged. Tables V and VI give the

diameters, in millimeters, of colonies of several mutants and their parents on two different media. It is evident that there were considerable differences in the rate of growth and the size of the colonies of different mutants. For example, on malt-sucrose agar the diameter of the parent, Minn. A, at the end of 11 days was 25 mm. while that of A4-6 and A11-1 was 19 mm. and those of A11, A13, and A14 were 49, 45, and 33 mm., respectively. It is obvious that some mutants grow much more slowly than their parents while others grow far more rapidly. (See Table VI.)

TABLE VI

RATE OF GROWTH OF MINN. A AND SOME OF ITS MUTANTS AS MEASURED BY THE DIAMETERS OF COLONIES ON TWO DIFFERENT AGAR MEDIA

Lines	Medium and diameters of colonies in mm.*	
	Potato dextrose + 0.5 per cent glycerine + trace of gentian violet	Potato dextrose + 1 per cent malt
Minn. A	15	25
A3-1	18	21
A4-2	10	24
A4-3	12	21
A4-6	15	19
A4-7	18	20
A8	18	21
A9	11	21
A10	8	22
A11	18	49
A11-1	17	19
A12	21	21
A13	..	45
A14	..	33

* The potato-dextrose agar was 4 per cent Difco; diameter of colonies listed in first column is the average of 2 colonies, 33 days old, and in the second column of 3 colonies, 11 days old.

There were remarkably consistent differences in the size of the colonies of a series of mutants from W. Va. A8-4, grown for purposes of comparison on potato-dextrose agar, potato-dextrose agar plus one-half strength Shive's solution, and litmus-lactose agar. The parent produced colonies with diameters of 45, 45, and 55 mm. on potato-dextrose agar, Shive's agar, and litmus-lactose agar, respectively, whereas A8-4-4 produced colonies with diameters of 50, 50, and 60 mm., respectively. In this particular case the diameter of the colonies of the mutant was 5 mm. greater than that of the parent on all three media. In other cases in this series the size of the colonies of certain mutants was approximately the same as that of the parent. These differences in rate of growth sometimes are observable only on certain media. For example, 13 mutants arose from W. Va. A8-5-1. One of these, W. Va. A8-5-1-5, produced two large mutants which looked almost identical, and were labeled A and B tentatively until it could be ascertained whether they actually were identical. Both were transferred

on the same day to two flasks each of potato-dextrose agar, potato-dextrose plus half-strength Shive's solution agar, and litmus-lactose agar. On potato-dextrose agar the two were so nearly alike that they might easily have been considered identical. On litmus-lactose agar they were fairly distinct in appearance but not in size. On the Shive's solution agar, however, the colonies were quite distinct, both in general appearance and in size. The colonies of A were 40 mm. in diameter, whereas those of B were 50 mm. This shows that it is necessary to consider the media on which the cultures are grown in determining rate of growth and size of colonies. A mutant may grow much more rapidly than its parent or other mutants on one medium, but the result may be the exact opposite on another.

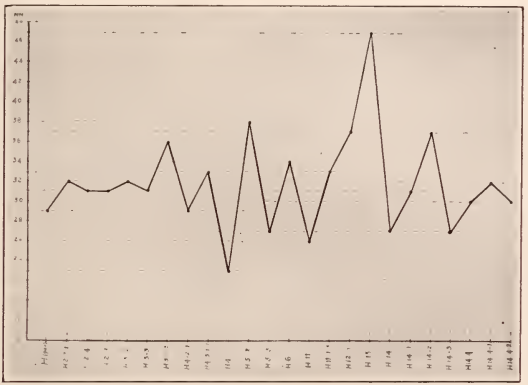


Fig. 4. Differences in the diameters of colonies of 23 mutants and their parent, Minn. A (designated in diagram by H), when grown on potato-dextrose-sucrose-malt agar.

The most extensive series of measurements were made on derivatives of W. Va. A8. It will be recalled that 220 mutants were obtained from a monosporidial culture of this line. Of these, 162 were distinct. When final observations were made on the 22C lines, the diameters of the colonies were measured. There were three flasks of each line, but only the average is given in Table VII. In most cases there was very little difference in the size of the three colonies of the same line, usually not more than 3 or 4 mm., altho in some cases there was greater variation. In general there was so little variability within the lines that the results seemed indicative of the genotypic tendency of the different lines to produce colonies of a certain size. The class inter-

vals, as will be seen from Table VII, are 8 mm. This seems sufficiently large because the fact had been determined by repeated observations that colonies of the same line seldom vary so much under uniform conditions.

TABLE VII

Size Classes of Colonies of 180 Mutants Derived from W. Va. A8, Grown on the Same Medium and under Same Conditions in Triplicate Erlenmeyer Flasks of Uniform Size

Class intervals in mm.	Number and percentage in classes	
	No.	Per cent
30-37	23	13
38-45*	82	45
46-53	61	34
54-61	14	8
Total	180	

* Parent falls into this class

Measurements are available for 180 of the lines. The line producing the smallest colonies was A8-5-12. These measured 28, 31, and 30 mm., respectively, an average of about 30 mm. That producing the largest was A8-5-1-2a-3, with colonies 61, 58, and 61 mm., an average of 60. The extreme range in size between the colonies of different mutants was 30 mm., the largest being twice the size of the smallest.

One usually thinks of mutants as having lost certain factors. However, some of this series have acquired growth factors or have lost factors that inhibit growth. This is evident when one considers that of the 180 lines compared, 82 produced colonies of the same size class as those of the parent, 23 produced smaller colonies, and 75 produced colonies larger than those of the parent, or 45, 13, and 42 per cent, respectively. It is possible that this does not represent exactly the true state of affairs. The more rapidly growing sectors are most conspicuous in a colony, and possibly a larger proportion of them were selected than of the smaller ones. Furthermore, in some lines of smut there is a tendency for the production of very small patch mutants, many of which probably never are isolated. Altho an attempt was made to select all that developed, regardless of size, it is likely that many mutant primordia are formed, but never become large enough to be observed clearly.

Many mutants derived from Minn. A and from W. Va. A8 have been grown repeatedly in different liquid media. There were decided differences in rate and amount of growth, as indicated by the mats sometimes formed on the surface, by the amount of flocculent material in the medium, and by the amount of sediment on the bottom of the flask. The differences in the character and amount of growth in the

various flasks were quite as sharp and great as similar differences in the growth of many species of bacteria in liquid media.

Elevation of colonies.—Not only are mutants characterized, to some extent, by the amount of radial growth of colonies, but also by their elevation. Colonies may be effuse, flat, raised, convex, pulvinate, capitate, umbonate, umbilicate, or combinations of several. There are types also that scarcely fall into any class. Thus in W. Va. A8-5-2-1 there is a tendency for part of the colony to separate from the remainder along a horizontal plane and to assume a somewhat irregular cupulate form, especially at low temperatures. This habit is peculiar to this particular line. The effuse and umbilicate types probably are the rarest, altho effuse colonies are common on poor media. It can be seen from Table IV that there are several types of elevation, even in a more or less random sample such as the lines listed in the table comprise.

Sometimes a particular type of elevation is so peculiar to colonies of a certain line that it alone almost suffices for identification. W. Va. A8-5-4 can be distinguished from many similar lines by the broadly umbonate character of its colonies, in which there is a broad, thick, central mound, often 25 mm. in diameter, surrounded by a flat zone of different color. (See Plate IV.)

Color of colonies.—There may be a wide range of color mutations in a monosporidial line. Albino mutants arise frequently in colonies of various colors; the color of mutants often but no always is lighter than that of the parents. There may be an intensification of color, or even a complete change, as well as a loss. (See Plate I.) Plate I, B shows one pure white sector, two dark ones with whi e margins, and several that are as dark as, or darker than, the parent. In Plate I, A are shown several sectors that are much darker than the parent.

Sectors or patches of several different colors often appear in duplicate colonies or even in a single one. In fact, almost any combination may occur. For instance, there appeared in duplicate pinkish colonies of W. Va. A8-4 sectors with the following colors: white, ivory, yellow, olivaceous, and black. In one pinkish colony of W. Va. A8-5 growing on potato-detrose plus Shive's solution agar there were 6 sectors colored as follows: white, cream-colored with a tinge of pink, grayish purple, olive, London smoke, and black. Minn. A14 was wood-brown and gave rise to sectors with the following colors: white, fawn, auburn, and pale vinaceous drab, the latter subsequently producing one of army-brown color.

The following colors were recorded for Minn. A and 6 of its mutants: Minn. A, pallid mouse gray; Minn. A1, seal brown; Minn. A2, vinaceous buff; Minn. A3-1-1-1, ecru drab; Minn. A10, blackish brown;

Minn. A11-1-2, white; Minn. A11-2, straw yellow. The following range of colors appeared among the 162 mutants from W. Va. A8; white, ivory, pearl gray, light buff, tan, light orange, deep orange, orange brown, and purple brown.

Color is not always uniform throughout a colony—there may be two or more color zones. This is well illustrated by W. Va. A8-5-7-1. (See Table IV and Plate IV.) The color may be masked somewhat by the production of aerial conidia that may give the surface a powdery or chalky appearance that may be so pronounced as almost to obscure the color underneath.

The color of colonies is, of course, subject to great variation under different environmental conditions. A culture, for example, may be cream-colored on one medium and almost black on another. Under similar conditions, however, color is fairly constant and quite characteristic of the mutant lines. This is well illustrated by the following observation. On September 10, 1928, one of the writers noted that colonies of W. Va. A8-5-4 were much darker orange brown on potato-dextrose agar than those of W. Va. A8-5-1. Soon thereafter the cultures of this series were given to another person for experiments on physiologic characters. The writer saw them again, quite by chance, on September 1, 1929, and again noticed the decidedly deeper color of A8-5-4. Upon looking at his original notes to see how the colors had been recorded a year previously, he found that they coincided almost exactly with his new observations, altho the cultures were on a slightly different medium.

The luster of colonies of different mutant lines may differ considerably, partly depending upon whether the colonies are sporidial or mycelial. Sporidial colonies may be either glistening or dull, while mycelial colonies naturally are dull, powdery, or cretaceous. Aerial conidia may be produced in either case. The result is likely to be a powdery appearance, thus modifying the color of the colony.

Surface characters of colonies.—It is clear from Plates II, III, and IV that the surface characters of colonies of different mutants may differ greatly. In the case of the mycelial type of growth the surface may be waxy, pubescent, cottony, felty, or finely or coarsely tufted. It may be smooth, or contoured, rugose, verrucose, bullate, or reticulate. In contoured colonies there again is considerable variation. In rugose colonies the folds may extend principally in one direction or in several. They may be simple or branched and anastamosing, giving all sorts of appearances from fine to coarse coral-like, sponge-like, or morel-like—some of which are very beautiful. Again, the size of the folds may vary. They may be large, small, or intermediate, and may be wide or narrow, and ridge-like rather than fold-

like. There also are all degrees of fineness and coarseness of the verrucose condition in colonies with this type of topography.

The marginal characters of colonies vary greatly with the different lines. In some cases the margin is entire, in others undulate, lobate, erose, lacerate, fimbriate, or floccose. Very often there is a distinct marginal zone in which the consistency, surface characters, luster, and color are quite different from those of the body of the colony. There may be a white border on deeply colored colonies, or it may be darker in color than that of the remainder of the colony. Sometimes there is a border of fine, silky hairs, ranging in width from two or three millimeters to a centimeter or more. Again, the border may be characterized by the presence of radial ridges or furrows of various shapes and sizes, whereas the remainder of the colony may have a quite different topography.

The surface of colonies may be uniform throughout or characteristically zoned. The zones may differ in topography or color, or both. (See Plate IV.) As all 8 lines shown in Plate IV are mutant derivatives of W. Va. A8, it is evident that the factors for zonation are subject to mutation. W. Va. A8-5-7-1 has several distinct color zones, but the topography is uniform throughout the colony, while in A8-5-4 the two zones differ in both color and topography. The same is true of A8-3-1, A8-5-5, and A8-5-3-2-5. It is interesting to notice that A8-5-4 and A8-5-5 appeared as sectors in the same colony of A8-5. Both have radial ridges in the marginal band, altho there are differences in their characters, but the color of the band in A8-5-5 is only slightly lighter than that of the remainder of the colony, while in A8-5-4 it is always much lighter, often becoming pure white.

Physiologic Characters

General statement.—Mutations often, perhaps almost always, involve some change in physiologic characters that are not so obvious as changes in general cultural characters. It was observed a number of times in studying the influence of media on frequency of mutation that certain lines of *U. zeae* changed the color of litmus media more than others, and there seemed to be some differences in ability to absorb dyes. On media containing litmus it sometimes was possible to pick out certain mutants readily by the color change produced by them, indicating that they were producing a reaction different than their parents.

Some preliminary experiments were made in order to determine differences in alkali production. Seven mutant lines were grown on slightly acid potato-dextrose agar to which was added a trace of brom-cresol-purple indicator. The production of alkali is indicated by a change in the color of the medium from yellowish to deep purple.

The results are summarized in Table VIII. It is evident that Minn. A3-1-2 produced alkali more rapidly and in larger amounts than A4-4-5 and A1-1. The differences were very striking during the first week or two of growth, but at the end of three weeks all of the mutants had produced a deep purple color. There was no obvious relation between the rate of growth and the rate of alkali production. For example,

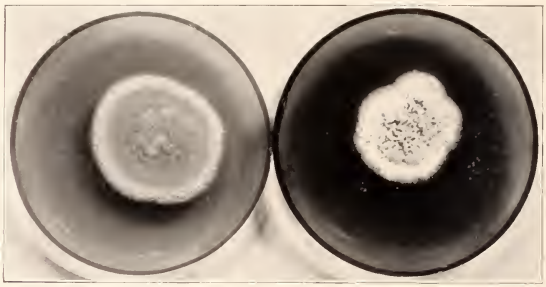


Fig. 5. Two mutants grown on potato-dextrose agar + brom cresol purple, showing differences in alkali production as indicated by intensity of color: *Left*, Minn. A1-1; *Right*, Minn. A3-1-2.

A3-1-2 grew slowly, but produced alkali rapidly, while A1-1 grew rapidly, but produced alkali slowly. The tests were repeated on potato-dextrose agar plus ½ per cent litmus solution, with similar results. (See Fig. 5.)

TABLE VIII

Rate of Growth and Alkali Production of Minn. A and Eight Mutants as Measured by Diameter of the Colonies and the Color Reaction on Potato-Dextrose Agar Plus a Trace of Brom-cresol Purple

Lines	Degree of alkali production*			Diameter of colony in mm.
	14 days	18 days	22 days	18 days
Minn. A	+	++	+++	41
A1	+	++	+++	45
A1-1	o†	o†	++±	42
A3	o	o†	++±	38
A3-1-2	+++‡	+++	+++	36
A4	o	+	++	36
A4-4-5	o	o†	++±	28
A10	±	+±	++	38
A3-1-1-2-1-1-1-3	±	±±	++	39

* o = none; + = alkali production, the degree being shown by number of signs; ± = slight.

† Purple under colony: no diffusion.

‡ Entire medium deep purple.

There also were marked differences in the ability of different lines to absorb indicators or dyes. For example, Minn. A4-4-5 absorbed brom-cresol purple, while A3-1-1-2-1-1-1-2 remained relatively free from it. There were similar differences in the absorption of color on media containing methyl red, methyl orange, gentian violet, and litmus.

Growth on standard bacteriologic media.—Because of these preliminary indications of physiologic differences between different mutant lines, it was decided to make a more intensive study of a few lines. The following, all derivatives from W. Va. A8, were selected: A8-1-1, A8-5-2-1, A8-5-3-2-5, A8-5-4, A8-3-1, A8-5-3-3-2, A8-5-5, A8-5-1-3-3, A8-5-1-2, A8-5-1, A8-5-1-6, and A8-5-7-1. First they were grown on standard bacteriological media to find out whether there were decided differences in behavior. The media were prepared according to the methods recommended by the Society of American Bacteriologists (16). The reaction of all media except milk was adjusted to pH 7 with NaOH, using brom-thymol blue as indicator.

All 12 lines grew slowly on solid media containing beef extract and peptone, attaining a diameter of only 3 to 6 mm. in two weeks. In virtually all cases the outline of the colony on beef extract media was irregular, the color white or creamy, and the surface either powdery or wet shiny.

In peptone broth and peptone alone there was considerable variety in the type of growth produced by the different mutants. All except one produced at least some surface growth, but there were considerable differences in the amount. This surface growth usually was present in the form of small particles of various sizes floating on the surface, rather than as a complete pellicle or ring. The surface growth of some mutants was moist on top while that of others was dry and powdery. In some cases the medium was turbid because of the presence of very fine aggregations of mycelium or sporidia; in others it was clear except for small flocculae. Usually there was some sediment, the amount varying with the different mutants. W. Va. A8-5-3-1-2 was unique in the fact that it produced no surface growth, and the liquid was perfectly clear except for the presence of abundant fluffy masses at the bottom of the tube.

A positive test for indol could not be obtained when the organism was grown on peptone. Neither was the odor of the broth nor of the peptone changed.

According to Levine and Carpenter (38), bacteria can be divided into three classes by their effect on gelatin: (1) those that do not hydrolyze; (2) those that partially hydrolyze and liquefy gelatin, with subsequent decomposition slow, if any; (3) those that completely hydrolyze and liquefy gelatin, with subsequent rapid decomposition.

Decomposition of the gelatin was based on formol titration of amino groups.

Ustilago zeae falls into the third class because all of the mutants studied reduced gelatin to a fluid of relatively low viscosity. There were, however, significant differences in the rates at which different lines caused liquefaction. Except in the case of two lines, liquefaction was complete a definite distance from the top, and the boundary between liquid and solid was very distinct for these lines. In Table IX are summarized the data for 12 lines grown for 12 days in triplicate flasks at 18°C. It is apparent by comparing the data in Table IX with those in Table XIV that, altho some mutant lines grew well at 18°C., they did not liquefy gelatin rapidly at that temperature. Evidently, therefore, temperature is not the limiting factor, but the differences in liquefaction are due to the differential ability of the smut lines to grow on gelatin and to produce gelatin-liquefying enzymes.

TABLE IX

TYPE OF GROWTH AND AMOUNT OF LIQUEFACTION PRODUCED BY MUTANT LINES OF W. VA. A8 GROWN FOR TWELVE DAYS ON GELATIN AT 18°C.

Mutants	Depth of liquefaction in mm.	Description of growth
A8-1-1	14-15	Medium growth on surface and at boundary between liquid and solid
A8-5-2-1	10	Medium growth on surface and at boundary between liquid and solid
A8-5-3-2-5	12-15	Abundant growth at surface and at liquid-solid boundary; tendency for mycelium to float in medium
A8-5-4	Very little	Surface growth slight
A8-3-1	11	Abundant surface growth; moderate growth at liquid-solid boundary
A8-5-3-3-2	Very little	Slight surface growth
A8-5-5	15	Moderate growth at surface and at liquid-solid boundary
A8-5-1-3-3	20	Liquefaction complete about 10 mm. deep and then a crater-shaped hole, 10 mm. deep
A8-5-3-1-2	38-40	Liquefaction nearly complete except for a little at the bottom and an irregular shaped piece 15 mm. long along one side.
A8-5-1	25	Growth moderate at surface and at liquid-solid boundary; boundary even except in one case where the submerged inoculum near the boundary started to grow
A8-5-1-6	15	Abundant growth on surface; medium growth at liquid-solid boundary
A8-5-7-1	15	Medium growth on surface and at liquid-solid boundary

Skim milk was first slightly coagulated by the cultures, probably by chymase, as there was no apparent change in acidity. The casein was digested later, leaving the milk translucent and light yellow in

TABLE X

Relative Rate of Digestion of Casein by Mutants of W. Va. A8 As Measured by the Size of the Colonies and of the Cleared Area After Having Grown on Milk Agar for Nine Days at Room Temperature

Mutants	Flask No.	Experiment 1		Experiment 2		Mutants	Flask No.	Experiment 1		Experiment 2	
		Diameter in mm.		Diameter in mm.				Diameter in mm.		Diameter in mm.	
		Colony	Clear area	Colony	Clear area			Colony	Clear area	Colony	Clear area
A8-1-1	1	14	36	18	41	A8-5-5	1	19	40	21	44
	2	10	30	15	36		2	19	40	21	43
	3	14	37	16	38		3	18	38	21	43
	4	..	..	15	36		4	..	..	21	43
A8-5-2-1	1	14	17	12	15	A8-5-1-3-3	1	20	45	21	43
	2	14	19	12	14		2	19	44	22	46
	3	15	17	12	14		3	19	44	22	44
	4	..	..	12	15		4	..	..	21	43
A8-5-3-2-5	1	19	40	*	*	A8-5-3-1-2	1	12	37	15	47
	2	21	42	23	47		2	12	37	*	*
	3	19	40	22	45		3	15	42	13	42
	4	..	..	21	44		4	..	..	15	47
A8-5-4	1	17	30	18	36	A8-5-1	1	17	41	20	44
	2	15	29	18	34		2	18	43	†	†
	3	17	31	17	31		3	19	43	20	44
	4	..	..	18	34		4	..	..	18	42
A8-3-1	1	19	41	22	43	A8-5-1-6	1	15	37	14	35
	2	19	42	21	41		2	14	37	14	35
	3	19	40	21	41		3	14	37	13	34
	4	..	..	21	41		4	..	..	14	35
A8-5-3-3-2	1	14	27	17	22	A8-5-7-1	1	14	38	18	39
	2	16	32	17	27		2	14	37	18	39
	3	14	27	18	29		3	14	37	17	36
	4	..	..	17	21		4	..	..	18	39

* Contaminated.

† No growth.

color. There was some surface growth in the case of all lines, but the amounts differed. Usually there was a ring pellicle at first, which later extended and covered the entire surface.

Digestion of casein.—It was observed in the foregoing experiments that different mutant lines digested casein at different rates. In order better to study these differences, milk was incorporated into a solid medium prepared as follows: 25 cc. distilled water plus 2½ per cent agar poured into 250 cc. Erlenmeyer flasks and sterilized under 15 pounds of steam pressure. While the agar was still hot, 15 cc. of milk was added to each flask, using aseptic technique. In order to avoid caramelizing the milk sugar and precipitating the albumins, the flasks were heated in an Arnold sterilizer for 20 minutes, after which they were taken out and allowed to cool. The resulting medium was pure white and homogeneous. The flasks were then inoculated in triplicate or quadruplicate with each mutant line. As early as one day after inoculation a yellowish translucent area began to appear around the colonies. This area approximated a definite circle in form and was of fairly definite size for each mutant line. Apparently the enzyme produced by the fungus diffused through the gel and evidently was produced in different amounts by different lines. The size of the colonies and of the digested areas are given in Table X. Results seemed to indicate rather clearly that there are decided differences in the ability of the different lines to digest the casein in the milk.

TABLE XI

Ratios of the Area of Milk Agar Digested by Mutants of W. Va. A8 to the Area of the Colony, Nine Days After Inoculation

Mutants	Clear area / Colony area		Mutants	Clear area / Colony area	
	Experiment 1	Experiment 2		Experiment 1	Experiment 2
A8-1-1	7.53	6.00	A8-5-5	4.44	4.24
A8-5-2-1	1.47	1.40	A8-5-1-3-3	5.26	4.49
A8-5-3-2-5	4.28	4.56	A8-5-3-1-2	8.95	10.02
A8-5-4	3.36	3.78	A8-5-1	5.54	5.04
A8-3-1	4.65	3.68	A8-5-1-6	6.52	6.28
A8-5-3-3-2	3.81	2.03	A8-5-7-1	6.93	4.64

This apparently is due to differential ability to produce proteolytic enzymes, as there was no correlation between the size of the colony and the surrounding area in which the casein was digested. (See Table XI and Plate V.) Amino acid determinations were made by the Vanslyke method and indicated that the protein actually was digested, but the comparative results obtained by the method were so variable that it seems possible that the digestion was not carried to the same stage by all lines.

Similar differences in the ability of certain fungi to produce en-

zymes have been noted by Currie and Thom (17), who showed that the ability of different strains of Penicillium to produce oxalic acid differed considerably. Oshima and Church (40) found that strains of *Aspergillus* of the flavus-oryzae group differed considerably in the amount of protease and amylase which they were able to produce, and Letcher and Willaman (37) showed that physiologic forms of *Fusarium lini* differed with respect to alcohol production.

Effect on sugars.—No gas was formed nor were there any apparent changes in pH when the cultures were grown on various pure carbohydrates as the only source of carbon. A liquid medium was used containing KH_2PO_4, KCl, $MgSO_4$, plus the particular carbohydrate to be tried, as recommended by the Society of American Bacteriologists (16) for use in detecting small changes in pH. The following carbohydrates were used: l-xylose, d-mannitol, d-levulose, and maltose. The medium was sterilized in the Arnold sterilizer in order to avoid changes in the sugars. Brom-thymol blue was used as indicator.

All 12 mutants grew well on each of the 4 carbohydrates, altho they grew slightly better on maltose than on any of the other 3. There was no visible change in the color of the indicator; neither did gas collect in the gas traps placed in the tubes.

Reduction of nitrates and iodine.—The ability to reduce nitrates to nitrites is characteristic of many bacteria and is used as one of the criteria of identification. In preliminary experiments with *U. zeae* grown on solid media containing KNO_3, it was found that if iodine was added to the medium several days after inoculation it was either absorbed or reduced near the mycelial mat, and no longer gave a blue color with starch. It was thought that this might be due to the reaction of the iodine with the nitrites formed in the medium. The following experiment was therefore made. Each of the 12 lines listed in Table XII was grown on 8 flasks of solid medium, 6 of which contained agar, starch, sucrose, $MgSO_4$, K_2HPO_4, and KNO_3. In the other 2 the KNO_3 was omitted.

Five days after inoculation the flasks containing the KNO_3 gave a strong test for nitrites with sulphanilic acid and alpha-naphthylamine. Ten days after inoculation, 2 cc. of a saturated solution of iodine in 50 per cent alcohol was added to 2 flasks of each line growing on the medium containing KNO_3, as well as to those which contained none. Colorless circular areas, of different size for each line, appeared on the KNO_3 media. When more iodine was added, the typical blue color reappeared, indicating that the iodine and not the starch was affected. The iodine was reduced slightly and slowly in the medium containing no nitrate. Thirteen days after inoculation iodine was added to the remaining 2 flasks of each line containing the nitrite.

Several lines, one in particular, caused vigorous reduction of the iodine, while others reacted very slightly. The result is hard to account for, but it is possible that some of the lines used up large amounts of nitrate for further growth. Comparative data are given in Table XII, and the appearance is shown in Plate VI. The results seemed to indicate that much of the reduction of iodine was due to nitrites produced by the fungus through the reduction of nitrates. Apparently another substance that causes the disappearance of the iodine-starch color is produced in small amounts, as indicated by the slow clearing of media containing no nitrates.

TABLE XII

Differences in Production of Iodine-Reducing Substances by Mutants of W. Va. A8, As Measured by Diameter of Colony and of the Clear Area

Mutants	Flask	Medium, treatment, and diameters of colonies in mm.					
		Iodine added 10 days after inoculation				Iodine added 13 days after inoculation	
		KNO_3 in medium Diameter in mm.		No KNO_3 in medium Diameter in mm.		KNO_3 in medium Diameter in mm.	
		Colony	Clear area	Colony	Clear area*	Colony	Clear area
A8-1-1	1	10	19	7	10	12	21
	2	9	19	7	10	14	26
A8-5-2-1	1	14	15	10	+	12	0
	2	10	11	10	+	12	0
A8-5-3-2-5	1	14	18	16	+	19	0
	2	13	17	16	+	19	0
A8-5-4	1	13	20	14	12	19	19
	2	13	18	13	11	19	19
A8-3-1	1	16	34	17	14	23	65
	2	14	27	15	13	24	72
A8-5-3-3-2	1	12	16	13	13	17	20
	2	13	18	14	14	18	21
A8-5-5	1	18	22	17	+	24	26
	2	17	21	18	+	25	27
A8-5-1-3-3	1	15	15	18	+	25	0
	2	15	15	18	+	27	0
A8-5-3-1-2	1	12	15	11	+	15	15
	2	12	13	13	+	16	16
A8-5-1	1	13	14	16	++	20	20
	2	..	..	15	++	20	20
A8-5-1-6	1	8	16	7	7	10	14
	2	7	15	8	8	9	12
A8-5-7-1	1	15	15	19	—	19	19
	2	14	14	19	—	18	18

* + = clear area barley discernible; ++ = stronger than + but still too small to measure; — = no action.

Fellenberg and Geilinger (21) state that *Aspergillus niger* is able to take up elemental iodine, but they attributed the action to a binding of the iodine by the proteins in the fungus. It is apparent, however, that the iodine-reducing substance produced by *U. zeae* is able to diffuse away from the culture through the agar gel.

The blue color gradually disappeared from uninoculated control flasks about 10 or 12 days after the iodine was added. It was thought that

this might be due to the fact that sucrose had become inverted when sterilized in the presence of K_2HPO_4. The action in this case, however, is too slow to compare with the above described phenomenon, which appeared within a few hours. Furthermore, the iodine would not disappear in a circular area under such circumstances.

It is significant that the rate of action of the different lines on iodine differs greatly. W. Va. A8-3-1 was outstanding in that it cleared the iodine much more rapidly than did the other lines and also left the medium with a reddish tinge after the iodine-starch color had disappeared. This condition was not observed in cultures of any other lines. While the results of these experiments cannot be explained fully, they do indicate clearly that there are quantitative physiologic differences between mutants of *U. zeae*.

Temperature relations.—It is known that physiologic forms of several fungi may have different temperature requirements. This has been shown by Edgerton (*20*) for forms of *Glomerella cingulata;* by Hursh (*27*) for forms of *Puccinia graminis tritici;* by Rodenhiser (*42*) for forms of *Ustilago nuda, U. tritici,* and *U. hordei;* and by Harter and Weimer (*25*) for strains of *Rhizopus nigricans.* Johnson (*28*) also reported that one form of *Helminthosporium gramineum* grew much better at low temperature than did another form of this species.

TABLE XIII

Effect of Temperature on the Rate of Growth of Mutants of W. Va. A8, As Measured by the Average Diameters of Duplicate Colonies Grown for 16 Days on Potato-Dextrose-Malt Agar*

Mutants	Temperature, and diameters of colonies in mm.†			
	10° C.	18° C.	26° C.	34° C
A8-1-1	4	19	13	16
A8-5-2-1	22	28	29	24
A8-5-3-2-5	7	21	26	24
A8-5-4	5	21	25†	6
A8-3-1	8	31	32	11
A8-5-3-3-2	10	24	35	19
A8-5-5	7	22	33	18‡
A8-5-1-3-3	10	25	31	28
A8-5-3-1-2	10	23	24	12
A8-5-1	6	23	29	15
A8-5-1-6	5	8	17	22
A8-5-7-1	6	19	33	18

* All flasks kept for 48 hours at room temperature, after which they were kept for two weeks at the various temperatures.

† Fractions counted as nearest whole number.

‡ Only one colony measured.

There were outstanding differences in the temperature requirements of the mutant lines of *U. zeae* listed in Table XIII. These lines were grown on potato-dextrose agar containing potato extract, 1 per cent dextrose, 1 per cent sucrose, and 1 per cent by weight of Trommer's malt syrup. Duplicate Erlenmeyer flasks, each containing 32 cc. of

this medium, were inoculated with each line, and kept at room temperature for 48 hours, after which they were placed at 10°, 18°, 26°, and 34° C., respectively. At the end of 14 days at these temperatures the diameters of the colonies were measured in two directions at right angles to each other, and the results averaged. This was particularly necessary in this case because of the fact that at some temperatures the outlines of colonies are irregular rather than circular. The results are given in Table XIII.

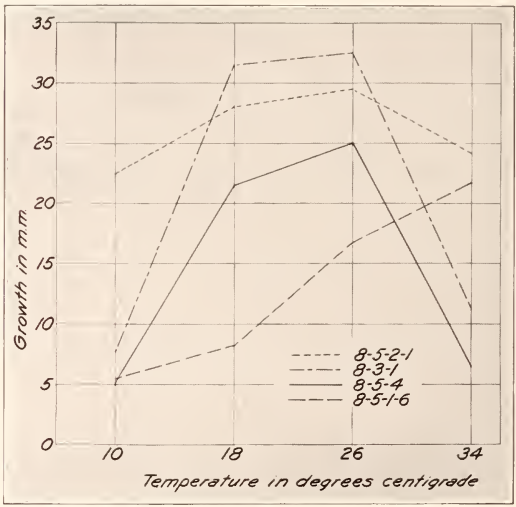


Fig. 6. Chart showing rate of growth as indicated by diameter of colonies of four mutants derived from *U. zeae* W. Va. A8, grown for 14 days at different temperatures.

It will be seen from Table XIII that W. Va. A8-5-2-1 grows well at a wide range of temperature, whereas A8-3-1 and A8-5-4 grew only very slowly at the extremes. A8-5-1-6 appears to have a higher optimum than any of the others. Not only was the size of colonies greatly influenced by temperature, but there were decided differences in the topography. There was a decided tendency for colonies of A8-5-2-1 and A8-3-1 to curl up away from the medium at the lower temperatures. At high temperatures, on the other hand, cultures of these lines were very closely appressed to the agar, and even cracked it in some cases and then grew into the cracks. (See Fig. 6.)

The results confirm the large number of miscellaneous observations to the effect that temperature profoundly affects the rate of growth, the final size and appearance of colonies, and that this effect varies with different mutant lines of *U. zeae*.

These experiments also furnish additional evidence that mutant lines of *U. zeae* differ in physiologic characters, some of the most outstanding of which are: ability to liquefy gelatin, digest casein, reduce nitrates and iodine; and in temperature relations.

Morphology of Sporidia

It already has been pointed out that there may be mutations in type of growth. Lines characterized by the mycelial type of growth may produce mutants which are predominantly sporidial and vice versa. As pointed out previously, there are all gradations between mycelial and sporidial types. It was noted also that the sporidia of some mutants differed decidedly from those of their parents and other mutants in shape and size, when grown under the same conditions. For example, Texas A is easily distinguished from Minn. A by its larger, blunt-ended sporidia. It seemed likely that there might be consistent differences in the morphology of sporidia of different mutants from the same parent. Therefore the question was investigated.

TABLE XIV

SIZE, SHAPE, AND COLOR OF SPORIDIA OF MINN. A AND THREE OF ITS MUTANT DERIVATIVES GROWN IN A SOLUTION OF 1 PER CENT GELATIN AND 2 PER CENT DEXTROSE FOR 14 DAYS

Lines	Length (in microns)	Character of surface growth and of sporidia
Minn. A	13.0	Sporidia regular, hyaline, usually fusiform; growth on medium, tan colored
Minn. A-3-1-2	9.7	Similar to the above, but sporidia usually more blunt; a distinctly white growth on the medium
Minn. A-4-4-5	13.7	Sporidia of two types, regular or irregular, frequently dumbbell in shape; growth slightly lighter in color than that of parent
Minn. A13	15.1	Sporidia mostly irregular, of various shapes, often simulating chlamydospores; growth on the medium dark

Three mutants, Minn. A3-1-2, Minn. A4-4-5, and Minn. A13, and the parent, 16 Minn. A, were grown in a liquid medium consisting of 1 per cent gelatin and 2 per cent dextrose in distilled water. The sporidia were examined after the cultures had grown for two weeks. The usual precautions were taken to select a random sample of sporidia for measurement, all of those encountered in passing from one end of the mount to the other being measured. It was considered that 98 spores would constitute a fair random sample of the populations studied. The results are summarized in Table XIV.

It seems evident from Table XIV that the sporidia of some of the

mutants of Minn. A differ from those of the parent and from those of other mutants. Those of Minn. A3-1-2 appeared to be shorter than those of the parent, while those of Minn. A13 appeared longer. The most conspicuous difference in morphology was the production of dumb-bell-shaped sporidia of Minn. A4-4-5. Minn. A13 produced sporidia which could easily be distinguished from those of the other lines because they were darker in color and frequently resembled immature chlamydospores. This experiment was repeated two months later under conditions similar to those of the first test. The results with respect to shape of sporidia corresponded closely with those obtained in the first experiment.

It is difficult to make experiments on morphology of sporidia under conditions that are rigidly enough controlled to justify drawing final conclusions. This is owing to the fact that many lines mutate so frequently that very careful precautions must be taken to exclude mutants from the cultures that are to be studied. This is especially true when the cultures are grown in liquid media, in which mutation cannot be observed directly. Nevertheless, the authors are convinced that consistent differences in the morphology of the sporidia come about as a result of mutation. Thus, sporidia of some of the mutants from W. Va. A8 were narrowly fusiform, others cylindrical, and others ovoid. The color, also, differed greatly under certain conditions. Sporidia of some lines were distinctly hyaline, others somewhat olivaceous, and still others almost fuliginous. As a matter of fact, one would scarcely recognize the sporidia of some mutants as those of *U. zeae*.

Pathogenicity and Sex

Sex and pathogenicity are to a considerable extent interdependent in *U. zeae*, as normal infection of the corn plant usually results from the growth of the dikaryophase in the corn tissues. According to Hanna (24), the haploid hyphae from germinating sporidia can penetrate the tissues; but ordinarily smut galls and chlamydospores are not formed unless two hyphae of opposite sex fuse and give rise to a binucleate mycelium (24, 44). Apparently most monosporidial lines, alone, cannot cause the disease, altho it will be shown in Part II of this bulletin that a few can. These monosporidial lines are the only entities that can be propagated independently and the only ones in which mutations in sex and pathogenicity can be studied. But so far it has been necessary to study both by observing the behavior of a line in combination with others. The study of the pathogenic characters of mutants would be simpler, therefore, if the pathogene were not predominantly heterothallic.

The problem is complicated further because there are more than two sexual groups, with varying degrees of sexual compatibility, that

is, multiple factors for sex; consequently it is difficult to know to what extent pathogenicity is merely a function of sex factors and to what extent a function of the physicochemical attributes of the haploid lines that unite to produce the pathogenic phase of the fungus. Gall formation is closely associated with chlamydospore production, and degrees of pathogenicity conceivably may depend largely on the complement of sex factors carried by the dikaryons and diplonts. On the other hand, haploid lines do differ in physiologic characters, and it seems probable that they may have varying degrees of virulence also. In addition, some lines mate perfectly well and produce normal chlamydospores without, at the same time, producing galls as large as those produced by other combinations of lines, thus furnishing evidence that pathogenicity is not solely a function of sex compatibility.

Whatever the ultimate factors for virulence may be, the degree of pathogenicity depends to a considerable extent on sex; hence changes in sex are accompanied by changes in pathogenicity. If a line that can unite readily with several other lines produced rapidly growing and prolific mutants that can unite with fewer lines, the potential pathogenicity of the pathogene has been decreased. The converse might also be true. Until more precise information becomes available, it seems best to consider sex and pathogenicity together.

Blakeslee (3, 4), Kniep (33), and Derx (18) observed change of sex (usually loss of sex factors) in *Mucor, Schizophyllum,* and *Penicillium,* respectively, after they had been grown for some time in artificial culture. This phenomenon is common in *U. zeae* also. Some of the most vigorous lines, sexually, have lost their power to unite with lines of opposite sex, but in every case the line in which such a change occurred was known to mutate frequently, at least under some conditions.

Christensen (13) stated that in the case of *Helminthosporium sativum* there were mutations in the pathogenic capabilities of certain forms. The mutants usually were less virulent than the parent, but some were more virulent. Stakman, Christensen, and Hanna (45), and Hanna (24) called attention to apparent change in sexual reaction and pathogenicity of some mutants of *U. zeae.* Further experiments were made by the authors.

The method of preparing inoculum and inoculating the plants was essentially that described by Stakman and Christensen (44). The lines were grown separately in a solution of 2 per cent dextrose and 1 per cent malt syrup. They were allowed to grow for two or three weeks before inoculations were made. The cultures were then mixed just before inoculation and the inoculum injected into the plants by means of a hypodermic syringe, as near the growing point as possible. The

controls were always inoculated in like manner with sterile nutrient solution and with monosporidial cultures.

Results of inoculations with certain mutant lines from Minn. A are summarized in Tables XV and XVI. It will be noted that there were pronounced differences in the parasitic power of some of the mutants, as compared with that of their parents and other mutants.

TABLE XV

RESULTS OF INOCULATING CORN IN THE GREENHOUSE WITH SEVEN MUTANTS OF MINN. A IN COMBINATION WITH THEIR PARENT AND WITH THREE OTHER MONOSPORIDIAL LINES OF KNOWN SEX*

Lines			No. plants inoculated	No. plants with galls	Severity of infection
Minn. A1-1	x	Minn. A	48	0	.....
do	x	Minn. F	0	..	.....
do	x	Minn. D	37	2†	Warts
do	x	Canada A	46	14‡	Slight; 2 large galls
Minn. A3	x	Minn. A	25	0	.....
do	x	Minn. F	25	20	Moderate
do	x	Minn. D	27	24	Moderate
do	x	Canada A	29	24	Moderate
Minn. A3-1	x	Minn. A	32	0	.....
do	x	Minn. F	0	..	.....
do	x	Minn. D	29	24	Heavy
do	x	Canada A	33	31	Heavy
Minn. A4-2	x	Minn. A	46	0	.....
do	x	Minn. F	58	56	Heavy
do	x	Minn. D	59	49	Moderate
do	x	Canada A	51	36	Moderate
Minn. A4-7	x	Minn. A	30	0	.....
do	x	Minn. F	0	..	.....
do	x	Minn. D	38	28	Heavy
do	x	Canada A	20	18	Heavy
Minn. A6	x	Minn. A	15	0	.....
do	x	Minn. F	0	..	.....
do	x	Minn. D	13	10	Moderate
do	x	Canada A	13	12	Heavy
Minn. A10	x	Minn. A	22	0	.....
do	x	Minn. F	0	..	.....
do	x	Minn. D	22	16	Light
do	x	Canada A	37	19	Light
Minn. A12	x	Minn. A	60	0	.....
do	x	Minn. F	53	45	Heavy
do	x	Minn. D	65	52	Heavy
do	x	Canada A	54	44	Heavy
Minn. A13	x	Minn. A	60	0	.....
do	x	Minn. F	60	57	Heavy
do	x	Minn. D	62	56	Heavy
do	x	Canada A	51	40	Heavy
Minn. A	x	all mutants	31	0	.....
do	x	Minn. F	62	39	Heavy
do	x	Minn. D	60	51	Heavy
do	x	Canada A	53	47	Heavy

* Four selfed lines of corn as well as Golden Bantam were inoculated but variations in degree of infection on the different lines were not very great. The results, therefore, are included together.

† Formation of chlamydospores doubtful.

‡ Two large galls near base of plant, perhaps due to contamination.

TABLE XVI

RESULTS OF INOCULATING GOLDEN BANTAM SWEET CORN AND GEHU CORN WITH MINN. A AND FOUR OF ITS MUTANTS IN COMBINATIONS WITH FIVE OTHER LINES OF *Ustilago zeae**

Lines	No. plants inoculated	No. plants infected	Degree of infection
Minn. A x Minn. F	15	15	Heavy
do x Canada A	16	14	Heavy
do x Minn. B	14	14	Heavy
do x Minn. C	..	..	.....
do x Minn. D	15	14	Heavy
Minn. A1-1 x Minn. F	12	0	.....
do x Canada A	16	2	Warts only
do x Minn. B	13	6	Moderate
do x Minn. C	15	0	.....
do x Minn. D	14	1	One large gall†
Minn. A3-1-1-2-1-1 x Minn. F	15	12	Moderate
do x Canada A	15	11	Moderate
do x Minn. B	18	12	Moderate
do x Minn. C	16	0	.....
do x Minn. D	17	16	Heavy
Minn. A4-4-5 x Minn. F	8	8	Heavy
do x Canada A	16	1	Light
do x Minn. B	11	9	Heavy
do x Minn. C	..	..	.....
do x Minn. D	18	13	Heavy
Minn. A13 x Minn. F	17	17	Heavy
do x Canada A	15	3	Light
do x Minn. B	17	16	Heavy
do x Minn. C	14	0	.....
do x Minn. D	16	11	Heavy

* Reactions of two varieties were approximately the same; the results therefore are combined.

† Gall near base of plant perhaps due to contamination.

It is apparent from Table XV that Minn. A unites normally with lines of opposite sex, Minn. F, Minn. D, and Canada A, producing heavy infection. Obviously, none of the mutants could do better on the lines of corn used in the experiment. Many did not do so well as the original parent, indicating a loss of virulence by mutation, which one would expect. But it seems clear that some mutants may be more virulent than their immediate parent. This is shown by the fact that Minn. A3 caused only moderate infection in combination with Minn. D and Canada A, while Minn. A3-1 caused heavy infection with these same lines. The virulence of this mutant exceeded that of its immediate parent, but not that of its "grandparent." This could hardly be expected of it, however, as the "grandparent" already had the maximum virulence for the variety of corn inoculated.

Mutants clearly may differ from each other in pathogenicity and sex, indicating that mutations in these characters occur (Table XV). One of the most striking changes that has been observed was in Minn. A13. This line produced heavy infection with Minn. F, Minn. D, and Canada A. (See Table XV.) When experiments were made

nine months later, however. Minn. A13 produced only light infection with Canada A. The experiments were repeated, but the differences remained constant. At present A13 is only weakly parasitic with Minn. F also, indicating a change in virulence, but not a complete change in sex factors.

Mutants were paired within their parents (Tables XV, XVII) and in no case did they unite, indicating that the sex had not changed completely by mutation. There are differences in virulence, however, as can be seen by a perusal of Tables XV and XVI.

TABLE XVII

Results of Inoculating Minn. No. 13 Corn with W. Va. A8 in Combinations with Six of Its Mutant Derivatives, and with Two of Its Mutant Derivatives and One Sister Mutant in Combinations with the Same Six Mutants

Lines	A8	A8-5	A8-5-1	A7
A8	..	..	0*	..
			15	
A8-5-1	0	0	0	0
	15	21	18	18
A8-5-1-1	0	0	0	0
	17	21	16	17
A8-5-1-2	0	0	0	0
	18	18	20	17
A8-5-1-6	0	0	0	0
	20	24	19	20
A8-5-1-7	0	0	0	0
	19	18	18	15
A8-5-1-8	0	0	0	0
	17	21	19	23

* Denominator = number plants inoculated; numerator = number plants infected.

In Table XVIII are recorded the results of inoculating Minn. No. 13 corn and Gehu with W. Va. A8-5-1, its immediate mutant, A8-5-1-1, and six mutants from the latter. It is apparent that A8-5-1-1-3a and A8-5-1-1-5a were less virulent than both of the above-mentioned on both varieties of corn inoculated. On the other hand, A8-5-1-1-6 seems more virulent on Gehu, both with Minn. E and Italy A1, than its immediate parent. The same appears to be true of A8-5-1-1-7. There are indications, therefore, that there may be a certain degree of specificity of certain combinations of smut lines for certain varieties of corn; and there is further evidence that mutants may be either more or less virulent than their parents.

TABLE XVIII

RESULTS OF INOCULATING TWO VARIETIES OF CORN WITH W. VA. A8-5-1 AND SEVEN OF ITS MUTANT DERIVATIVES IN COMBINATIONS WITH MINN. E AND ITALY A1

Lines	Smut lines, corn varieties, and degree of infection*			
	Minnesota E		Italy A1	
	Minn. 13	Gehu	Minn. 13	Gehu
A8-5-1	6^3 M ± / 9	5 L ± / 5	2^8M ± / 11	1 / 10
A8-5-1-1	11 M ++ / 11	5 S ± / 5	1^2 M ± / 9	4 S + / 5
A8-5-1-1-2a	11 M to L +++ / 11	6 M to L +++ / 6	11 M to S ++ / 13	6 S to M + / 7
A8-5-1-1-3a	4^6 M + / 13	3^4 VS − / 7	2^8 S ± / 11	0 / 5
A8-5-1-1-5a	5 S + / 5	8^7 S to M ± / 15	4 S ± / 7	11 S to M + / 13
A8-5-1-1-6	5 M ++ / 6	13 S to L +++ / 13	6 M +++ / 6	13 M to L +++ / 13
A8-5-1-1-7	3 M +++ / 4	11 VL ++ / 12	5 S ++ / 7	13 L +++ / 14
A8-5-1 1 8	6 M ++ / 7	12 M + / 13	S ± / 7	8 S to M + / 11

* The denominator denotes the number of plants inoculated and the numerator the number on which galls were produced . The exponent numbers indicate the number of plants on which incipient galls were produced.

L = large galls.
VL = very large galls.
M = medium sized galls.
S =small galls.
VS = very small galls.

+++ = very many galls.
++ = many galls.
\+ = moderate number of galls.
± = few galls.
− = very few galls.

Table XIX summarizes the results of inoculating Golden Bantam corn with W. Va. A8 and a number of its mutant descendants in combinations with several other lines. A8-1-1 is at least equally as virulent as the parent, and possibly more so, but the other three are less virulent in all combinations. It appears that A8-5-5 and A8-5-3-1-2 are considerably less virulent than W. Va. A8, altho they have not completely lost the power to unite with those lines with which A8 unites, except perhaps with Italy A1. Even A8 does not form a virulent combination with Italy A1. It will be noted that A8 is only weakly parasitic with Minn. C, also. W. Va. A8-5-3-3-2 seems to be entirely unable to unite with any of the lines with which it was mated, apparently being entirely devoid of the necessary sex factors.

The results again show that some mutant lines form virulent combinations with certain other lines and only very weakly parasitic combinations with others. They also show that mutation may result in changes in sexual and pathogenic powers. A8-5-5 and A8-5-3-1-2 may have lost certain factors for sex, as relatively few chlamydospores

were produced, or it may be that the dikaryophytes were unable to grow well in the tissues of the host because factors for physiologic vigor were absent. Either interpretation would seem plausible, but further studies must be made before drawing final conclusions. W. Va. A8-5-3-3-2 probably has lost the sex factors of the parent and has become sterile, or it is of different sex, as it failed to form chlamydospores or to produce clearly visible indications of infection with any of the lines with which it was paired.

TABLE XIX

Results of Inoculating Corn with W. Va. A8 and Four of Its Mutant Derivatives in Combinations with Eight Other Monosporidial Lines

Lines	Smut lines and degree of infection*				
	W. Va. A8	W. Va. A8-1-1	W. Va. A8-5-5	W. Va. A8-5-3-1-2	W. Va. A8-5-3-3-2
Minn. A	11 M + / 12	10 M ++ / 10	7 I to S − / 11	3 I to S − / 13	0 / 15
Minn. C	4 I to S ± / 12	4 I to S ± / 11	2 I / 14	3 I / 15	0 / 11
Minn. E	11 M + / 12	13 L +++ / 13	5 I / 10	0 / 11	0 / 14
Spain A	11 L +++ / 12	13 L +++ / 14	4 I to S − / 11	3 I to S − / 11	0 / 13
Italy A1	9 I to S ± / 11	8 M + / 10	0 / 11	0 / 12	0 / 11
Minn. A13 x Minn. F.5†	11 L +++ / 11	13 M ++ / 14	7 I / 11	6 I to S − / 10	0 / 13
Minn. A13 x Minn. F.8	15 M +++ / 15	12 L +++ / 13	3 I / 11	6 I to S − / 13	0 / 10
Minn. A13 x Minn. F.10	16 L +++ / 17	11 L +++ / 12	5 I to S − / 11	0 / 14	0 / 10

* The denominator denotes the number of plants inoculated and the numerator the number on which galls were produced.

L = large galls.
M = medium sized galls.
S = small galls.
I = incipient galls.

+++ = very many galls.
++ = many galls.
\+ = moderate number of galls.
± = few galls.
− = very few galls.

† Minn. A13 x Minn. F.5, etc., = fifth sporidium from the cross indicated.

Mutations in sex and pathogenicity evidently occur commonly and may result either in gain or in loss of factors, apparently for both characters, altho loss seems more common than gain. It appears evident that there may be various degrees of loss of virulence and sexual power in mutants from the same monosporidial line. The authors are of the opinion that, in *U. zeae* at least, the loss of virulence in culture is due to mutation, as the changes of this nature that they observed were sudden, not gradual. Furthermore, there is positive evidence that there is not necessarily a correlation between the virulence and

sexual vigor of a monosporidial line and the length of time it has been grown in artificial culture. Minn. E and Minn. F have, for example, been in culture for four years and have lost neither sexual nor pathogenic potency. These lines do not mutate frequently; consequently it is relatively easy to maintain their purity. But this is not true of the lines that mutate freely, and it was in such lines that the authors observed most of the so-called loss of sex and pathogenicity. Sex mutations would be observed less easily than mutations resulting in such easily observable characters as color, type of growth, etc. In fact, the only way to determine them would be by inoculations, unless the mutants happened to differ in appearance. Using ordinary mycological culture routine, there is no assurance that a culture will remain the same after several successive transfers as it was at the beginning. Care must be taken to transfer each time from the parent, and not from the parent plus one or more mutants, or from mutants alone. Changes in pathogenicity of a culture do occur; but not by gradual degradation due to growth on artificial media, at least so far as the authors' observations go. Unless necessary precautions are taken to transfer continually from the original line, the culture is likely to comprise several lines after relatively few "cultural generations," and none of these may be the same as the original, which easily may have been lost in transferring cultures by ordinary methods. A true explanation of loss of pathogenicity might be that the pathogenic line was lost in the shuffle, not that the pathogenic line lost its pathogenicity.

It seems clear that physiologic forms or monosporidial lines differ in general cultural characters, including rate of growth, direction of growth, type of growth, surface characters of colonies, and color. They differ also, to a certain extent, morphologically, physiologically, in tendency to mutate, and in sex and pathogenicity. Differing, as mutants do, in these characters, it follows that the factors for the characters are subject to mutation. It seemed desirable, therefore, to ascertain somewhat more precisely than was possible from general observations the environmental conditions under which mutations occur most frequently.

FACTORS AFFECTING MUTATION

There was abundant evidence from numerous experiments and observations that environment exerts a profound influence on the phenotypic characters of *U. zeae*. There was no evidence of slow, cumulative, heritable changes. Lines did not gradually lose their color, virulence, or similar characters; neither did they gradually acquire any new capabilities as a result of growing under different conditions. When changes did occur, they occurred suddenly and presumably by mutation; but there was abundant observational evidence that environmental

factors affected the frequency of mutation. Therefore, experiments were made for the purpose of obtaining data along this line.

Nutrients[5]

Christensen (13), Brown (8), and others have shown that the amount and kind of culture medium may affect the frequency of mutation. As general observations indicated that this was true also of *U. zeae*, experiments were made on the effect of nutrients. In one series seven monosporidial lines were grown on six different agar media. The results are given in Table XX. There were indications that the kind of medium affects the frequency of mutation; therefore more detailed experiments were made.

TABLE XX

THE NUMBER OF MUTANTS THAT DEVELOPED IN SEVEN MONOSPORIDIAL LINES OF *Ustilago zeae* ON SIX DIFFERENT AGAR MEDIA IN TRIPLICATE FLASKS FOR EACH MEDIUM

Lines	Medium and number of mutants*						
	I	II	III	IV	V	VI	Total
Texas A	0	0	0	0	0	0	0
Minn. A	6	8	3	8	1	0	26
Minn. G	2	0	0	0	0	0	2
Hungary A	..	..	..	0	0	0	0
Spain A	..	..	..	2	4	0	6
W. Va. A8	..	..	..	5	3	12	20
Minn. E	4	0	0	1	0	0	5
Totals	12	8	3	16	8	12	59

* Triplicate flasks were used throughout except for those listed in Column III in which duplicate flasks were used.

Media used:

I. 3.5 per cent Difco potato-dextrose agar
II. 4 per cent Difco potato-dextrose agar plus 2 per cent sucrose
III. 4 per cent Difco potato-dextrose agar plus 0.5 per cent glycerin and trace of gentian-violet
IV. 1.2 per cent Difco potato-dextrose agar plus 1 per cent litmus
V. Half-strength Shive's solution plus 3 per cent dextrose plus 1.2 per cent agar
VI. Half-strength Shive's solution plus 3 per cent lactose plus 1.2 per cent agar.

The following lines of *U. zeae* were used: W. Va. A8-5-1, Texas A, Canada A, and Minn. A. These lines were grown first on an agar medium containing various sugars without any mineral salts. The cultures attained considerable diameter but were always colorless and effuse, scarcely half the thickness of ordinary writing paper. Increasing the concentration of sugars did not stimulate more normal growth. Neither was there much difference in the growth on dextrose, sucrose, or a combination of both. No mutants appeared on the plain sugar agars. The addition of small amounts of $MgSO_4$ resulted in the production of more normal colonies, altho they were still colorless, and

[5] These experiments were repeated by M. B. Moore and J. M. Walter while the bulletin was in press. The results in general agreed closely with those recorded here.

no mutants appeared. The addition of small amounts, .006 per cent, of calcium nitrate or sodium nitrate, however, produced a profound effect. The colonies grew faster, became larger, and were much thicker than those grown on agar containing sugars only. In addition, numerous sectors were formed when the nitrate salts were added to the medium. Increasing the percentage of nitrate salts did not result in greater frequency of mutation but seemed to have the opposite effect. For example, the medium containing .012 per cent calcium nitrate appeared to inhibit growth as well as mutation. (See Table XXI.)

TABLE XXI

THE NUMBER OF MUTANTS THAT APPEARED IN FOUR MONOSPORIDIAL LINES OF *Ustilago zeae* ON VARIOUS NUTRIENT MEDIA

Medium	Sectors					Notes
	W. Va. A8-5-1	Texas A	Canada A	Minn. A	Total	
Agar 1.5%	0	1	0	0	1	
Sucrose 1.5%, agar 1.8%	0	0	0	0	0	
Sucrose 3%, agar 1.8%	0	0	0	0	0	Growth effuse. Culture of considerable diameter but very thin. Almost colorless
Dextrose 3%, agar 1.8%	0	0	0	0	0	
Sucrose 1%, dextrose 1%, agar 1.8%	0	0	0	0	0	
Peptone 1.5%, dextrose 1.5%, agar 1.8%	0	0	3	2	5	
Peptone 3%, agar 1.8%	0	0	0	0	0	
Sucrose 1.5%, agar 1.8%, $MgSO_4$ .018%	0	0	0	0	0	Slightly healthier growth than those on sugar alone
Sucrose 3%, agar 1.8%, $NaNO_3$ .006%	5	1	0	4	10	Growth more rapid. Colonies longer and several times thicker than those on sugar media
Sucrose 3%, agar 1.8%, $NaNO_2$ .006%	0	3	16	17	36	
Sucrose 1.5%, agar 1.8%, Ca $(NO_3)_2$ .012%	0	0	5	3	8	
Sucrose 3%, agar 1.8%, $(NH_4)_3PO_4$ .012%	0	0	0	0	0	Colonies only about half as large as those on nitrate media but much thicker; yellowish to orange
Sucrose 3%, agar 1.8%, $(NH_4)_2PO_4$ .012%	0	0	0	0	0	
Sucrose 1.5%, agar 1.5%, $(NH_4)_3PO_4$ .006%	0	0	0	0	0	
Sucrose 1.5%, agar 1.5% K_2HPO_4 .01%	0	0	0	0	0	Very similar to those grown on sugar media
Sucrose 1.5%, agar 1.5%, Ca $(NO_3)_2$ .005%, $(NH_4)_3PO_4$ .005%	0	0	0	0	0	Colonies slightly larger than those on nitrate media
Half-strength Shive's solution, agar 1.5%	0	6	0	2	8	
Carter's medium*	0	0	0	0	0	

* Carter's medium; 20 gm. dextrose; 10 gm. peptone; 10 gm. NH_4NO_3; 5 gm. KNO_3; 2.5 gm. K_2HPO_4; 2.5 gm. $MgSO_4$; 0.1 gm. $CaCl_2$; 20 gm. agar; 1000 cc. dist. H_2O.

No mutants appeared on sucrose agar plus different amounts of ammonium phosphate. The diameter of the colonies on such media was only about half that of those on the nitrate media, altho they were much thicker, some of them being 5 mm. or more in thickness. Color production was very pronounced, the colonies being yellowish to orange. Transfers were made from these thick, bright-colored colonies to sugar agars and the same type of growth resulted as had appeared originally, indicating clearly that there had been merely a temporary modification. Sectors were never seen on the phosphate media.

Experiments then were made to ascertain whether the addition of small amounts of ammonium phosphate to the media on which sectors were produced abundantly would inhibit their formation. To the medium consisting of 1.5 per cent agar, 1.5 sucrose, and .006 per cent calcium nitrate was added .006 per cent of ammonium phosphate. The colonies that developed were almost identical in appearance with those grown on the sugar media plus ammonium phosphate alone, altho they were slightly thicker and somewhat larger. No sectors appeared, the addition of the ammonium phosphate apparently having counteracted completely the effect of the nitrate in stimulating mutation.

It was suspected that the PO_4 radicle was responsible for the type of growth which developed when $(NH_4)_3PO_4$ was added to the media. To ascertain whether this was true, potassium phosphate was substituted for ammonium phosphate. The colonies grown on this medium differed greatly from those produced when the ammonium salt was used. The colonies were almost colorless and very effuse, resembling those produced on the sugar media. No sector formation was noted. This suggested that the PO_4 radicle was not responsible for the type of growth that developed when $(NH_4)_3PO_4$ was used as a constituent of the medium.

Temperature

From general observations and experiments there was evidence that certain lines of *U. zeae* mutated much more at certain temperatures than at others. This also is true of *Helminthosporium sativum* (14). Experiments therefore were made to determine the effect of temperature. W. Va. A8-5-1, Texas A, Canada A, and 17 Minn. A were used, with the results as summarized in Table XXII. Four separate experiments were made. In the first one the four lines of smut were grown in 4 flasks each at 8, 15, 20, 26, and 31° C. Sectors appeared only at 26° and 31° C. In the subsequent experiments tests were not made at 8° C. because the fungus failed to grow appreciably at that temperature. The results were consistent in all four tests, so it seems fairly certain that, in the lines tried, mutations either occur or become observable only at the higher temperatures. This is apparent from

the summaries given in Table XXII. There were no sectors in colonies grown at 8° and 15°, only one in all those grown at 20°; eight at 26°, and eighteen at 31° C.

TABLE XXII

EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF MUTANTS IN FOUR MONOSPORIDIAL LINES OF *Ustilago zeae*, GROWN ON FOUR DIFFERENT MEDIA, IN QUADRUPLICATE FLASKS FOR EACH MEDIUM

Medium	Temperature, degrees C.	Forms used and number of mutants produced by each*				
		W. Va. A-8-5-1	Texas A	Canada A	Minn. A	Total
Sucrose 10 gms., malt ext. 5 cc., agar 9 gms.	8	0	0	0	0	0
	15	0	0	0	0	0
	20	0	0	0	0	0
	26	0	0	1	0	1
	31	0	0	1	3	4
		0	0	2	3	5
Sucrose 10 gms., malt ext. 5 cc., agar 9 gms.	15	0	0	0	0	0
	20	0	0	0	1	1
	26	0	0	0	1	1
	31	1	1	5	0	7
		1	1	5	2	9
Sucrose 10 gms., $Ca(NO_3)_2$ .006%, agar 9 gms.	15	..	0	0	0	0
	26	..	0	2	0	2
	31	..	0	3	0	3
			0	5	0	5
Shive's solution agar	15	..	0	0	0	0
	26	..	4	0	0	4
	31	..	4	0	0	4
			8	0	0	8

Light

Fairly extensive tests to determine the effect of light on the frequency of mutation were made with the same smut lines that were used in studies on the effect of temperature, but the results were inconclusive. Half of the cultures were kept in constant light and half in constant darkness, but very few sectors developed, probably because the cultures were kept at 20° C., which seems too low to stimulate mutation.

DEGREE OF CONSTANCY OF MUTANT CHARACTERS

The same monosporidial lines may vary tremendously under different environmental conditions. As a matter of fact, virtually every cultural character by which lines are recognized is subject to extremes of variation. This is true of size of colony, type of growth, consistency, elevation, topography, zonation, and marginal characters. Even under conditions which permit fairly extensive growth, the variability

may be so great that one easily could conclude, on the basis of cultural characters on solid media, that the same line actually was several different lines. Some of the lines are more variable than others, but all are subject to considerable variability. As it often would be quite impossible to recognize the same line grown under different conditions, it is very important that standardized conditions be used to make comparisons of cultures grown at different times.

Fig. 7. Differences in the rate of radial growth of mutants and their parent, Minn. A (designated by H in diagram), when grown on two different media: *A*, potato-dextrose agar (measurements made on the 11th day); *B*, potato-dextrose agar + trace of gentian-violet (measurements on 33d day).

The composition of the medium may have a profound influence on the appearance of colonies. The amount of moisture, very slight differences in the composition and the amount of medium used, may affect the appearance of colonies considerably. The effect of media is shown clearly in Plate VIII, which shows Minn. A3-1-2 on three different media. It will be noted that the surface on potato-dextrose agar is decidedly convoluted but there are radial ridges near the margin, with fine white mycelium at the edge. On potato agar the growth is much more effuse; therefore the convolutions are less distinct, and the marginal ridges almost imperceptible. On 1 per cent glycerine potato agar there are a few convolutions near the center of the colony and deep radial furrows with only a faint suggestion of radial ridges on a few of the lobes near the edge of the colony.

Several series of lines from W. Va. A8 were grown at various times under uniform conditions on potato-dextrose agar, potato-dextrose plus half-strength Shive's solution agar, and litmus-lactose agar. A close study was made of the characters of W. Va. A8-4 to A8-4-5, inclusive, and of A8-5 to A8-5-5, inclusive. In all cases it was possible to distinguish the different lines on any one of the media alone, but the characters of the same line on the three media were quite different. The colonies on litmus-lactose agar, for example, were on the average considerably larger than those on the other two media, but the growth was more effuse. There was a tendency for most characters to appear most sharply defined on the potato-dextrose plus Shive's solution agar. The characteristic color was generally most distinctive; the characteristic topography of the surface was most clearly defined; and the marginal characters were usually more outstanding. In some of the lines there was a tendency toward the production of a black color. This was always deepest on the agar containing Shive's solution. In lines characterized by rugose, contoured, or reticulate topography, the most pronounced development occurred on this medium. The reticulations were always larger and coarser and the radial ridges were almost always more sharply defined. These characters were almost entirely suppressed on litmus-lactose agar, altho in some cases there were indications of their occurrence. It is noteworthy, however, that when colonies grown on one medium were transferred again to another medium, they then assumed the distinctive characters of the particular line on that particular medium, indicating that there was no gradual fixing of characters because of growth on a particular kind of medium.

Effect of temperature on the appearance of colonies is shown fairly well in Plate IX, which pictures colonies of four lines grown at 26° C. and 18° C. (10° for A8-5-2-1). It will be noticed that colonies of W. Va. A8-5-2-1 resemble each other at the two temperatures, but the margin is more nearly entire at 26° C. At 10° C. it is quite irregular

and decidedly raised. The most characteristic difference is the fact that at 10° C. there is a tendency for part of the colony to grow away from the medium, forming a cup-shaped structure near the center. At 26° there are two well-defined zones in the colonies of A8-5-4, a central mound which is only slightly contoured, and a marginal band lighter in color and characterized by the presence of mid-sized, branched radial folds which intertwine somewhat at the base. At 18° C. there is virtually no zonation and the radial ridges are considerably suppressed, altho there are a few radial furrows. The colony of A8-5-7-1 at 26° is flat, smooth, and distinctly zonate. On the other hand, at 18° it is convex to umbonate, verrucose to rugose, and not zoned. It will be noted that the topography of the two colonies of A8-5-1-3-3 tends to be approximately the same, altho it is more pronounced at the higher temperature. The shallow radial folds produced near the margin of the colony at the higher temperature are entirely absent at the lower temperature. Furthermore, the surface of the colony at 26° C. was somewhat waxy, while at the lower temperature it was fine and felt-like. In most cases the colonies were smaller at 18° than at 26°, altho in at least one instance they were equally large at both temperatures.

Despite the fact that there is great phenotypic variability in the same lines of *U. zeae*, it is remarkable that they seem to be so constant for given conditions, except for mutation. There has been no evidence whatever, in the authors' studies, that a permanent change is induced by environmental conditions, except in so far as they stimulate mutation.

The concept of the "educability" of fungi apparently still is held by some mycologists. Loss of virulence of cultures, loss of factors for color production, and loss of sporulating ability have repeatedly been explained on the basis of degradation resulting from artificial culture. In *U. zeae*, the only changes of this nature that the authors have detected were clearly explicable on the basis of mutation, not on the basis of degeneration because of saprophytic nutrition. Mutability could easily account for much of the so-called educability, as pure lines of a very mutable fungus are difficult to maintain. One could scarcely guarantee the homogeneity of a culture which had been grown in culture for several months or a year without precautions to avoid transferring mutants along with the parent. The authors have observed hundreds of times that small, indistinct mutants may be very numerous on agar slants. It would be relatively easy to transfer these mutants during routine transfers. Furthermore, as pointed out previously, mutants may appear as indistinct sectors or patches on the colony. What assurance could there possibly be that dozens of these mutants were not transferred unless precautions were taken to avoid it?

It seems to the authors that the best method of insuring the purity of cultures of a mutable fungus is to grow it in flasks containing a

medium on which mutation does not occur commonly. Even then precautions must be taken to transfer from the parent colony and not from the parent colony plus one or more of the mutants. Brown (8) has called attention to the danger of transferring mutants and permitting them to accumulate in routine cultures unless similar precautions are taken. If stock cultures are carried in test tubes, it is desirable to grow them on a relatively poor medium for mutation and transfer frequently. Even then there is no assurance, in the case of a fungus like *U. zeae*, that the population of the cultures will remain homogeneous.

From all experiments and observations made by the authors they can only conclude that *U. zeae* is extremely unstable genetically, but not in the sense imputed by such words as educability, adaptation, and Abkultur. They have repeatedly, in fact thousands of times, observed the appearance of new lines, but only by mutation and hybridization.

It has been claimed that the phenomenon of reversion tends to prove that so-called mutants are not in reality true mutants. In *U. zeae* reversions to the parental type do occur, but in a relatively small percentage of cases; and then the evidence is that they occur by mutation.

Despite the frequency of mutation, the authors have grown many lines in culture for periods of time ranging from a few months to three years, without observing any inexplicable change. In fact, some of the cultures, principally those that mutate only very infrequently, have been grown constantly on artificial media for four years without alteration of their essential characters. The following experience is illustrative of this fact. During the summer of 1928 one of the authors made comparative studies of W.Va. A8-5-1 to W.Va. A8-5-5, inclusive. His final notes on some of these mutant lines were taken on July 4, 1928, and on the others, September 10, 1928. He then gave the cultures to one of the other authors for experiments on physiologic characters and did not see them again until September 1, 1929. Then he recognized the numbers in a temperature series and looked up his original notes to see how the descriptions corresponded with the characters apparent at that time.

One line, W. Va. A8-3-1, arose in a slightly pinkish colony of A8-3 as a large fan-shaped sector which was characterized by conspicuous radial folds and an olivaceous color, both of which were entirely lacking in the parent colony. The mutant was grown on several different media for comparison with the parent. The outstanding characters of the mutant on potato-dextrose agar at that time were its rather coarse growth, the distant and pronounced radial folds, and a mouse-gray to olivaceous color, obscured somewhat by a whitish-powdery superficial growth, and a mycelial margin of medium texture. On September 1,

the colonies on potato-dextrose-maltose agar grown at 26° C. were as nearly identical in appearance with those grown on a slightly different medium, at room temperature, a year previously as could be determined from the author's notes and memory. On inquiry it was found that this particular line had been transferred at least twenty times during the year; had been subjected to varying conditions of nutrition and temperature; and had produced at least one mutant. Nevertheless it was virtually identical in appearance a year later. Stock cultures of the mutants were then compared with stock cultures of the parent and it was obvious that the characteristic folds of the mutant were quite apparent and would serve to distinguish it readily from the parent, even on fairly old agar slants.

Another of the cultures in this series was W. Va. A8-5-1. On potato-dextrose agar, September 10, 1928, the size of the colonies was recorded as 50 mm., and the outstanding characters were described as follows: "Most of the colony distinctly convoluted, with a border about 1 cm. wide in which there are distinct radial ridges rather unique in the fact that they are shaped like a tuning fork; color of the central area of the colony grayish, the remainder gray-buff, and the marginal area a clear buff orange." It so happened that the colonies on potato-dextrose-maltose agar on September 1, 1929, were exactly 50 mm. in diameter, the central area was grayish, the remainder gray buff; the radial ridges were precisely as had been described a year previously; and the color of the marginal area in which they occurred was again a clear orange buff. W. Va. A8-5-4 was described September 10, 1928, as having a decided central mound 20 mm. in diameter and grayish buff with a tinge of purple in color on potato-dextrose agar, delicate pink on potato-dextrose plus Shive's solution agar, with a broad margin of rather fine, radial lines or ridges. On September 1, 1929, the central mound and the marginal characters were as described a year previously, altho the color was somewhat deeper. Furthermore, a note was made on September 10, 1928, that the color of A8-5-4 on potato-dextrose plus Shive's solution agar was deeper orange than that of A8-5-1. Precisely the same observation was made on September 1, 1929.

The characters mentioned above appear to be due to genetic factors, as they tend to appear under conditions that are in the least favorable for the growth of the organism. They can be altered or suppressed temporarily but always tend to reappear when the organism is grown under fairly normal conditions. The above are merely a few observations which convince the authors that the mutant lines are different genotypically and not merely phenotypically. It may be objected that the differences between mutants and their parents may be due to changes in the cytoplasm rather than in the nucleus, that they are, therefore,

somatic rather than genotypic changes. It is true that thus far the criterion of heritability has been satisfied only for asexually propagated mutants. It has been pointed out, however, that mutants often differ from parents in sex factors. This surely would seem to involve a change within the nucleus. Furthermore, there is evidence that factors for mutant characters are inherited through sexual reproduction as well as asexual propagation.

A study of the f_1 lines from crosses between lines of *U. zeae* indicates that there are multiple factors for most of the characters discussed in the foregoing pages, and that the organism is highly heterozygous for most characters. This complicates breeding tests.

It seems clear, however, from Table XXIII that the change in type of growth of at least one mutant from that of the parent is genotypic. The f_1 lines[6] from Minn. A x Minn. F, both mycelial types, were mostly mycelial. But those from Minn. F x Minn. A13, a sporidial mutant from Minn. A, were mostly sporidial. That Minn. A12 and Minn. A13, sister mutants from Minn. A, carry different genetic factors for growth type is indicated by the fact that most of the f_1 lines from Minn. A12 x Minn. D were predominantly mycelial. From Minn. A13 x Minn. D, on the other hand, there were 14 mycelial, 7 intermediate, and 11 sporidial types. This furnishes evidence that Minn. A13 differs from its parent, Minn. A, and from its sister mutant A12, in genetic factors for growth type. The change, therefore, would seem to be genotypic in nature—true mutation.

TABLE XXIII

GROWTH TYPE OF f_1 LINES FROM CROSSES BETWEEN CERTAIN LINES OF *Ustilago zeae*

Cross and growth type of parents	Number of f_1 lines of different growth types		
	Mycelial	Intermediate	Sporidial
Minn. A (mycel.) x Minn. F (mycel.)	13	1	1
Minn. A13 (sporid.) x Minn. F (mycel.)	4	2	7
Minn. A13 (sporid.) x Minn. D (mycel.)	14	7	11
Minn. A12 (mycel.) x Minn. D (mycel.)	12	0	1

DOES TRUE MUTATION OCCUR IN USTILAGO ZEAE?

The authors have used the term mutation to designate the phenomenon resulting in the sudden and fortuitous origin of the new lines of *Ustilago zeae* discussed in the foregoing pages. Some microbiologists and geneticists may, and probably will, object to the use of the term in this connection. Possibly we have not proved mutation.

[6] The sporidia on the promycelia of chlamydospores resulting from a cross can be considered the gametes of the F_1 individuals. They are therefore designated f_1, and cultures resulting from single sporidia are designated f_1 lines.

Whether we have depends largely on the concept of what mutation really is. The word has been used rather loosely, perhaps, in the literature of microbiology. The authors have used it to denote the sudden appearance of new lines of *U. zeae*, which apparently are not the result of normal segregation nor of abnormal environmental conditions, and the mechanism of whose origin is not, and possibly cannot be, known. Possibly the authors have used the term in a too liberal sense; that depends, of course, on one's opinion of what the word should connote.

There probably is no universal agreement, even among geneticists, as to the precise meaning of the word mutation. Baur (*2*, p. 339) defines it as follows: "Erbliche Verschiedenheiten zwischen den Eltern und ihren Nachkommen—auch den vegetativ entstandenen—welche nicht auf Bastardspaltung beruhen, sondern welche andere Ursachen haben." If this definition is accepted, the authors think they have proved the case beyond reasonable doubt for *U. zeae*. The changes that occur certainly persist through many asexual generations and evidently also through sexual fusions. They apparently are not the result of hybrid segregation, altho the authors can furnish no guarantee that there is not an unsuspected, or at least undetected, segregation of factors after sporidia have been formed on the promycelium, where segregation of sex factors and of those for cultural characters certainly usually occur. It is just such uncertainty as this that probably provoked Brierley's expressions of agnosticism in his philosophical essays on mutation in micro-organisms and in the interpretation of his own results (*3*, *6*, *7*).

Brierley (*7*) maintains that the phenomenon described as mutation in micro-organisms cannot be equated with mutation in higher plants and animals. His opinion is that it is premature to use the term in mycological studies when there is evidence only of changes of unknown genetic causation. Because of the ignorance of genetic constitution and chromosomal relations in fungi, Brierley thinks there can be no assurance of genetic purity even in monosporous lines. He points out that a monosporous line may be heterozygous and therefore has no greater genetic value "than a single-seed or a single-tuber culture derived from a probably or possibly cross-bred higher plant of unknown genetic constitution" (*7*, p. 724). He states further that no geneticist would accept such material as a sound basis for experimental work unless by breeding experiments he could analyze its genetic constitution and obtain progeny homozygous for the character he wished to investigate. This has not yet been possible for fungi. Attention is called also to the fact that monosporous fungal lines may be heterokaryotic, hence genetically impure, as a result of sexual fusions or anastamosing of

vegetative hyphae. The chance assortment of nuclei in cell division might then result in the appearance of "new strains." Brierley does not deny the possibility of mutation in fungi but states definitely that he is of the opinion that no single case has been described which satisfies the criteria essential to proof.

It is unfortunate that words must be used to describe phenomena and express concepts, because few words can be used with mathematical precision. If a scientific term meant precisely the same thing to all investigators, there undoubtedly would be fewer arguments—and probably less literature to read. After all, what most likely is needed is accurate descriptions of phenomena and clear-cut distinctions between those that are different. In the present case the difficulty seems to be that we do not know sufficiently well, at least in microbiology, just what the mechanisms are by which so-called mutations are brought about. The word mutation often has been used to designate sudden and unexplained changes of more or less permanent nature in the observable characters of micro-organisms. Blakeslee (3, 4), La Rue (35), Christensen (13), Leonian (36), Christensen and Stakman (15), and Stakman, Christensen, and Hanna (45) used the word mutation more or less in this sense. Stevens (46), Brown (8), Chaudhuri (12), Mitter (39), and others use the word saltation, while Burgeff (10), Caldis and Coons (11), and others use the term variants. Still others speak of modifications or "Dauermodifikationen." Caldis and Coons, for example, are of the opinion that the "achromatic variations" which they studied in several different fungi "represent semi-permanent variations which are different from the parent form somatically rather than genetically. These are the dauer-modifications of Jollos ————————." It would seem to be a pertinent question whether mycologists have used these different terms to describe essentially identical phenomena or whether the phenomena described under the various terms really were different. It is beyond the province of this bulletin, however, to subject to critical scrutiny all of the literature dealing with the so-called mutations in fungi.

That variations occur in fungi is a fact that even the most skeptically minded probably will accept as a platitude. But what is the nature of these variations? Ramsbottom (41), Brown (8, 9), Christensen (13), and many others have pointed out that environmental factors may profoundly affect the appearance of a fungus. As Ramsbottom (41, p. 40) expresses it, "Further investigations, such as those of Matruchot thirty years ago, showed that when certain media were used a fungus might have its facies so altered that by a systematist it would be placed in another genus. It is now one of the best known features of culture work that the form of a fungus can be altered in

all sorts of ways by the use of different media." With this statement most mycologists will agree heartily and most of them probably will agree that these purely temporary variations or modifications are not inherited even through vegetative propagation. Fungi may be shocked into looking queer by subjecting them to extraordinary stimuli, but they ordinarily regain their composure very soon after the stimuli have been removed. These ordinary, non-heritable variations certainly do not explain what have been called mutations. Possibly these statements would be so generally accepted as to be superfluous. But there still are some students of fungi who apparently believe in "educability"—the transmission of characters acquired as a result of environmental influences.

If fungi are "educable," many of them are very refractory subjects. At least that is the judgment of the two authors of this bulletin who have tried long and arduously to make fungi appreciate the benefits of education. We do not deny that cultures may be so weakened by maltreatment that their recuperative powers are taxed somewhat, but the pathogenic fungi to which we have tried to give opportunities for leading a different life have refused to do so. We can only conclude that the potentialities of fungi, like those of higher organisms, are determined by their genes, and the only way in which their potentialities can be changed is by changing their genes. Which means that fungi are the product of the interaction of genetic and environmental factors, just as higher organisms are, and that when heritable changes occur they come about as the result of hybridization or mutation. Possibly "Dauermodifikationen" may occur, as they apparently do in protozoa, but the authors do not believe they are dealing with this type of phenomenon in *U. zeae*, altho Goldschmidt (22) seems to be of the opinion that the preponderance of evidence indicates that "mutations" which he observed in *U. violacea* were of the nature of "Dauermodifikationen."

Jollos (29) described "Dauermodifikationen" in Paramecium. He was able to induce tolerance for arsenic by growing non-tolerant lines of Paramecium in a medium containing it. This acquired ability persisted through many asexual generations, but was lost immediately following conjugation and was eventually lost also in vegetatively propagated lines after they had been grown for some time on arsenic-free media. More persistent "Dauermodifikationen" were induced by calcium salts, inasmuch as the modification, reduction in rate of division, persisted not only through many asexual generations (about nine months in some cases), but also through two successive conjugations. One line, for example, reverted to normal after the second conjugation, but required several months longer for reversion when propagated asexually. Jollos considers this a case of "Dauermodifikation" also, as he does not believe the germ plasm was changed. His concept of this

phenomenon seems to be that a line can adapt itself temporarily to certain environmental conditions and retain its acquired ability for some time after the removal of the stimulus that brought it about. Reversion, loss of the acquired characters, is gradual as long as the organism propagates asexually, but it is either immediate or relatively rapid when sexual reproduction intervenes.

Jollos describes also what he considers a true mutation. He obtained a line of Paramecium comprising individuals that were characteristically smaller and more tolerant of high temperatures than the parental line. The new line retained its characteristics for more than a year in normal culture and also after repeated conjugations. Jollos therefore considers that it is justifiable to consider this a case of true mutation, a genotypic change. He thinks there are three categories of variations: ordinary modifications, "Dauermodifikationen," and mutations. Granting the correctness of his conclusions, the question arises as to whether the same type of phenomenon which he describes as "Dauermodifikation" occurs in fungi, and whether the so-called saltations or mutations fall into this category.

Baur (*2*, pp. 61-62) suggests that the adaption of many bacteria to particular animal or plant hosts and the specialization of certain rust fungi for certain hosts may possibly be of the nature of "Dauermodifikationen." The authors are firmly of the opinion that this is not true for the rust fungi, as one of them has devoted many years to a study of the question and is forced to conclude that physiologic forms of rust fungi are remarkably constant, as long as they are propagated asexually.[7] Uredinial lines of *Puccinia graminis*, Pers., on which most of the studies have been made, are dikaryophytes and may be heterozygous, but there is no evidence of segregation or chance assortment of nuclei; and cultures derived from a single urediniospore are dikaryotic clones with certain infective capacities that do not become permanently modified as a result of host influences. On the other hand, there is very strong evidence that the physiologic forms arise by mutation and hybridization, altho mutations apparently occur much less frequently than in many other fungi.

The conjugate nuclei in the dikaryophase of *Puccinia graminis* may have different factors for pathogenicity and sex; and the pathogenic capabilities of a uredinial line or physiologic form may be the component of the factors in the two nuclei, that probably were derived from two haploid lines. In this respect the aecial and uredinial stages of the rust would correspond with the dikaryophase of *U. zeae* and other smut fungi—with the difference that in the smuts the haplonts can propagate independently and the dikaryophytes do not reproduce as

[7] For a brief summary of the evidence and references to the original papers see No. 43 in the bibliography.

such, while in the rusts the haplonts cannot multiply independently, and the dikaryophytes can and do propagate independently. The physiologic forms or parasitic strains of *P. graminis*, therefore, are clones which owe their parasitic peculiarities to two conjugate nuclei containing different genes, but all the evidence obtained by the senior author and his associates supports the view that the forms differ genotypically and not merely phenotypically. These forms may be, and often probably are, heterozygous and many types of segregates may result after sexual fusion, but that is precisely what is expected. The teliospores are the true diplonts and one may expect the same sort of segregation on a teliospore promycelium as on a promycelium of a chlamydospore of *U. zeae*. The authors cannot believe that the forms are at all comparable with "Dauermodifikationen," and they are of the opinion that anyone who examines the published evidence or makes the experiments will agree.

Brown (8) was unable to obtain evidence of slow cumulative change in *Fusarium*. The saltations which he described appeared sporadically, suddenly, and without any apparent cause. They did appear in greater numbers on certain media than on others, but Brown offers a perfectly logical explanation for that. The same thing was true of the mutants described by the authors for *U. zeae*. And why should not mutations in fungi occur more frequently under some conditions than others? The mass of data on mutations induced in higher organisms by X-rays and radioactive substances should furnish sufficient supporting evidence, if it were needed, that mutation occurs more often under some conditions than others. But there is a decided difference between the gradual and temporary acquisition of a "purposeful" character, as in the case of Jollos' "Dauermodifikationen," and the sudden appearance of new lines that may or may not be better adapted to the conditions under which they were produced.

Caldis and Coons state that nutrients, light and darkness, and reaction of substratum were not controlling factors leading to production of the fungal variants they studied. Nevertheless they reach the following conclusions (11, p. 215), "It is the opinion of the writers that the variants dealt with in this paper represent rather semi-permanent variations which are different from the parent form somatically rather than genetically. These are the 'dauer-modifications' of Jollos (29), which perhaps by drying of the mycelium and lack of nutritional connection with the substratum, have become changed from the normal. With respect to certain attributes they may be looked upon as attenuated forms. Instead of being new species they are rather to be looked upon as cultures lacking certain physiological powers. They resemble, in some respects, the Abkulturs of Fusaria and the so-called attenuated cultures of bacteria and fungi. They may have arisen from cells whose

protoplasm has been poisoned, or perhaps affected by some unknown biological factor, and which are tardy in recuperation until supplied with the necessary conditions. This study illustrates that the criteria established for proof of the educability of fungi are necessary and that what may seem plausible evidence of the establishment of new forms in pure cultures needs careful and protracted investigation." This may be the correct explanation for the phenomena observed by Caldis and Coons, but it does not seem probable that it would explain some of the new entities of *Mucor genevensis* described as mutations by Blakeslee (3), nor the variants of *Phycomyces nitens* described by Burgeff (10). Blakeslee describes a "dwarf mutant" that lost the power to produce sporangia, to produce zygospores hermaphroditically, and did not unite sexually with plus and minus races of dioecious species. This mutant remained constant from 1913 until 1920, when Blakeslee published his statement regarding it. Some of Blakeslee's mutants reverted to the parental type and might be considered by some, therefore, as "Dauermodifikationen." But the suggested explanation for reversion given by Burgeff (10) for *Phycomyces nitens* and by Blakeslee for his Mucors seems more reasonable. Burgeff considers that mutation affects only some of the nuclei in *Phycomyces,* which is multinucleate. He calls these variants mixochimeras, in which the more rapid division of normal nuclei would account for reversions. Blakeslee thinks that his reverting mutants also may be mixochimeras.

What we have called mutations in *U. zeae* conceivably might be due to one of the following: (1) ordinary variation or modification; (2) "Dauermodifikation"; (3) mutation; (4) the isolation of individual lines from a mixed population; (5) the chance assortment of nuclei in the budding of possibly heterokaryotic sporidia; (6) ordinary segregation; (7) extraordinary segregation. That they are not ordinary modifications is perfectly clear from the fact that the characters of the new lines are long persistent. The authors do not believe that their mutations are analogous to Jollos' "Dauermodifikationen," because there is no gradual fixing of certain characters as a result of certain stimuli, followed by loss of the characters when the stimuli are removed. The new lines arise suddenly, sporadically, frequently in some lines, seldom in others. The new characters are persistent but may or may not enable the organism better to adjust itself to certain conditions. Very often factors for rate of growth, color, sex, pathogenicity, etc., seem to be lost, but in other cases there seems to be a gain. Possibly the authors are using "Dauermodifikation" in a too narrow sense, but, as they understand it, the essence of the concept is that it is a modification induced by definite environmental stimulus, that the modification persists for a limited time after the stimulus is removed and then is gradually lost if the organism multiplies asexually, and suddenly lost in sexual repro-

duction. The change is presumed to be in the cytoplasm, and possibly to a lesser extent in the nucleus. In the case of *U. zeae* the change often is in the nucleus, as the new lines may be quite different from their parents in sexual powers. Neither the cause of the change nor the nature of the change in *U. zeae* seems analogous to that which Jollos observed in Paramecium

If it will be agreed that the preponderance of evidence is against the probability that the new lines of *U. zeae* are either ordinary modifications or "Dauermodifikationen," there remain the possibilities that they are merely lines isolated from a mixed population, or that they are segregates. As the changes occur in great numbers in monosporidial lines, the possibility of isolation from an originally mixed population is ruled out. Before considering the other two possibilities it may be well to review what is known about the nuclear phenomena in *U. zeae*.

The chlamydospore nucleus typically is the result of karyogamy. It is a fusion nucleus, and is therefore diploid. On germination the spore produces a promycelium, and the diploid nucleus undergoes division. A second division occurs, resulting in the formation of four nuclei which become distributed in the promycelium. Usually three septa are then laid down, resulting in the formation of a four-celled promycelium, each cell of which contains one nucleus. Four sporidia then form, one on each cell of the promycelium. During this process the nucleus in the promycelial cell again divides, one daughter nucleus going into the sporidium and one remaining in the promycelial cell, which can, therefore, produce successive crops of sporidia. The sporidia are typically uninucleate. It has been shown by Hanna (24) that two of the sporidia usually are of one sex and two of another, altho sometimes they may belong to four sexual groups. Furthermore, the individual sporidia on the promycelium may produce colonies of different color and type of growth; hence there has been segregation of factors for sex, for color, and for type of growth, in one or both of the nuclear divisions in the promycelium. That is, reduction division has occurred. The sporidia, then, are haplonts, potential gametes; and monosporidial lines are haploid or gametic clones, as in most other Ustilaginaceae (34). That this probably is true is indicated by the fact that a number of times the authors have isolated several sporidia from monosporidial lines and compared them in respect to cultural characters and sex. The resulting lines behaved alike.

If monosporidial lines are in reality gametic clones it would be justifiable to consider the variant sectors that arise in colonies of these clones as true mutations, provided they result from a genotypic change. The authors think they do, as the general cultural and physiological

characters are constant—except as they may again change suddenly, presumably as a result of mutation. And, what is more important, the changes evidently affect the nucleus—the germ plasm, as is indicated by changes in sexuality. There is evidence, also presented in this paper, that at least some mutant characters, type of growth for example, persist through the sexual stage. Therefore it would seem that the criteria for mutation have been satisfied reasonably well. The authors freely admit that nothing is known about chromosomal behavior, if indeed definitely organized chromosomes exist; hence the evidence for reduction division in the promycelium is necessarily circumstantial. Possibly there is segregation of some kind in the process of budding of sporidia in sporidial cultures or in cell division in mycelial cultures, but, if there is, it would seem to be unusual, as only a very few individual sporidia or mycelial cells in proportion to the enormous number in a colony differ from the rest. The authors admit, also, the possibility of heterokaryosis, altho the sporidia appear to be characteristically uninucleate and to arise from uninucleate cells of the promycelium.[8] An explanation of the appearance of sectors on this basis would appear to be rather far-fetched in a fungus like *U. zeae* with its rather definite alternation of haploid, dikaryo, and diploid phases. The possible validity of such an explanation for the appearance of so-called mutants would seem to be greater in the case of fungi whose cells are typically multinucleate. If the so-called mutations in *U. zeae* are not due to gene changes or chromosomal aberrations, it seems more probable that they are due to some sort of unknown segregation.

The authors consider the evidence good, however, that changes, heritable both through asexual propagation and sexual reproduction, occur in gametic clones of *U. zeae;* that the changes are not due to segregation; and therefore are mutations.

SIGNIFICANCE OF MUTATION

Ustilago zeae comprises an indefinite number of haploid lines differing from each other in many characters. There seems to be no good reason why these lines should not be considered physiologic forms, as some of the most conspicuous differences between them involve physiologic characters. The authors are not disposed to urge the acceptance of this viewpoint, however; possibly it is just as well to use the term "line." Most of these lines are unisexual; hence hybridization is prerequisite to normal infection, except occasionally, as shown in Part II.

[8] Since the manuscript was written M. B. Moore and J. M. Walter have made an extensive study of the nuclear condition in several monosporidial lines. The sporidia and hyphal cells were almost universally uninucleate. The very few sporidia in which there were two nuclei were budding or about to bud. The senior author studied the slides thoroly and is convinced that the sporidia and hyphal cells in the lines studied are definitely uninucleate.

As the unisexual lines are very numerous and differ in many characters, large numbers of new genotypes may result from hybridization. Add to this the fact that mutation is so common, and it is not surprising that there are so many lines.

U. zeae seems to be extraordinarily variable and mutable. Most of the other smut fungi investigated seem to be far less mutable. Despite the enormous amount of work done with *U. violacea,* very few mutations have been recorded for this species. And, in extensive experiments with *U. tritici, U. nuda, U. hordei, U. levis,* and *U. avenae,* Rodenhiser (42) observed mutation only in *U. hordei* and *U. avenae,* and rather rarely in them.

The extreme variability and mutability of *U. zeae* is interesting in itself and emphasizes the statements of Leonian (36) and Brown (9) regarding the caution necessary in creating taxonomic systems of fungi that vary and mutate extensively. It seems probable that some of the peculiar behavior attributed to fungi in culture, loss of virulence for example, may often be due to mutation, or, if one prefers a more noncommittal statement, to the production of variants or saltants. Mutation seems also to be significant in the broader aspects of the problem of pathogenicity in *U. zeae.*

The fact that some mutants are adapted to growing well at a wide range of temperature suggests one way in which mutation may be important in epidemiology. Those lines that tolerate wide extremes of temperature are likely to be most prevalent. Under certain conditions they may develop well, while those lines with more rigidly fixed temperature requirements may develop very poorly or not at all. Temperature, then, may determine the prevalence of certain lines in different regions or in the same region in certain seasons.

Mutation may result in the development of lines with different physiologic and sex factors, and there is strong evidence that the lines differ in pathogenicity. The corn smut pathogene, therefore, comprises a large number of parasitic strains. The possible implications of this fact in the control of the disease by immunization are quite obvious. Christensen and Stakman (15) already have called attention to this fact. Much more work must be done to determine the pathogenic capabilities of combinations of mutant lines of *U. zeae* and of the nature of resistance in corn before final conclusions can be drawn. We know, however, that there are numerous parasitic strains, that new ones are being produced by mutation and hybridization, and that these facts may have important practical implications, but how important must be determined by future investigations.

SUMMARY

1. *Ustilago zeae* comprises an indefinite number of monosporidial or haploid lines that differ from each other in so many physiologic characters other than sex that they might well be considered physiologic forms.

2. Numerous sectors arise in cultures of most monosporidial lines of *U. zeae*. "Patch mutants" also may occur, but less frequently than sectors.

3. Some lines mutate abundantly, while others seem to do so rarely. From one line the authors isolated, within a year, 220 mutants, 162 of which were different. From another line 70 were obtained. A few lines have never been observed to mutate; others have been grown in culture for long periods of time without mutating; then they suddenly began to mutate.

4. Mutants may differ from their parents in type of growth, consistency of colonies, direction of growth, size of colonies, elevation, color, and surface characters of colonies.

5. Mutation often affects physiologic characters. Some of the outstanding physiologic differences between mutants were: ability to liquefy gelatin, digestion of casein, reduction of nitrates and iodine, and rate of growth at different temperatures.

6. The morphology of sporidia of different mutant lines may be different.

7. Pathogenicity and sex are closely interrelated in *U. zeae*. The pathogene seems to be predominantly heterothallic, the fusion of lines of opposite sex usually being prerequisite to infection. Therefore, the pathogenicity of combinations of lines only can be studied in most cases, altho it is shown in Part II of this bulletin that some monosporidial lines can cause infection alone.

8. It is not known to what extent pathogenicity is a function of sex factors and to what extent of physiologic factors. That it is not a function of sex only is indicated by the fact that certain lines may unite to form chlamydospores without producing large galls, while others may produce large galls.

9. Mutations in sex occur. Some mutants seem to have lost completely the sex factors that enable their parents to unite with other lines.

10. Many mutants seem to have lost factors for pathogenicity while others seem to be more virulent than the immediate parent.

11. Nutrients and temperature appear to affect the rate of mutation. In a series of experiments on the effect of nutrients, no mutants were observed on sugar media nor on sugar media plus $MgSO_4$ or phosphates. One appeared on plain water agar, a few on peptone-dextrose agar, and a considerable number on sugar media plus nitrates.

The largest number of mutants appeared in cultures grown at fairly high temperatures.

12. There seems to be good evidence that what the authors have designated mutations are in fact true mutations. The variant sectors or patches arise in monosporidial lines, which appear to be haploid clones; the new characters persist through asexual propagation, and there is evidence that at least some of them persist through sexual fusions. The change, therefore, appears to be genotypic.

13. The results with *U. zeae* suggest that some of the remarkable variability attributed to fungi in culture, loss of virulence, change in sex, and loss of sporulating ability, for example, often may be due to unobserved mutations.

14. Mutation would seem to be very important in the corn smut problem, especially in epidemiology and breeding for smut resistance.

LITERATURE CITED

1. Bauch, R. Untersuchungen über die Entwickelungsgeschichte und Sexualphysiologie der *Ustilago bromivora* und *Ustilago grandis*. Ztschr. Bot. **17**:129-177. 1925.
2. Baur, E. Einführung in die experimentelle Vererbungslehre. 5te u. 6te Auflage. Berlin, 1922.
3. Blakeslee, A. F. Mutations in mucors. Jour. Hered. **11**:278-284. 1920.
4. ———. Sexuality in mucors. Science **51**:375-382, 403-409. 1920.
5. Brierley, W. B. Some concepts in mycology—an attempt at synthesis. Trans. Brit. Mycol. Soc. **6**:204-234. 1919.
6. ———. On a form of *Botrytis cinerea*, with colourless sclerotia. Trans. Roy. Soc. London, Ser. B, **210**:83-114. 1920.
7. ———. Discussion on mutation of species. Brit. Med. Jour. **2**:722-726. 1922.
8. Brown, W. Studies in the genus *Fusarium* IV. On the occurrence of saltations. Ann. Bot. **40**:223-244. 1926.
9. ———. Studies in the genus *Fusarium* VI. General description of strains, together with a discussion of the principles at present adopted in the classification of *Fusarium*. Ann. Bot. **42**:287-304. 1928.
10. Burgeff, H. Untersuchungen ueber Variabilität, Sexualität und Erblichkeit bei *Phycomyces nitens* Kunze. Flora, (n.s.), **7**:259-316, 1914, and **8**:353-448. 1915.
11. Caldis, Panos D., and Coons, G. H. Achromatic variations in pathogenic fungi. Papers Mich. Acad. Sci., Arts and Letters **6**:189-236. 1926.
12. Chaudhuri, H. A description of *Colletotrichum biologicum*, nov. sp., and observations on the occurrence of a saltation in the species. Ann. Bot **38**:735-744. 1924.
13. Christensen, J. J. Physiologic specialization and parasitism of *Helminthosporium sativum*. Minn. Agr. Exp. Sta. Tech. Bull. 37. 1926.
14. ———. The influence of temperature on the frequency of mutation in *Helminthosporium sativum*. Phytopath. **19**:155-162. 1929.
15. ———, and Stakman, E. C. Physiologic specialization and mutation in *Ustilago zeae*. Phytopath. **16**:979-999. 1926.

16. Committee on Bacteriological Technic. Manual of methods for pure culture study of bacteria. Published by the Society of American Bacteriologists, 1926.
17. Currie, J. N., and Thom, C. An oxalic acid producing *Penicillium*. Jour. Biol. Chem. **22**:287-293. 1915.
18. Derx, H. G. Heterothallism in the genus *Penicillium*. Trans. Brit. Mycol. Soc. **11**:108-112. 1926.
19. Eddins, A. H. Pathogenicity of multisporidial and monosporidial cultures of *Ustilago zeae* (Beckm.) Ung. (Abst.) Phytopath. **19**:91. 1929.
20. Edgerton, C. W. Effect of temperature on *Glomerella*. Phytopath. **5**:247-259. 1915.
21. Fellenberg, Th. von., und Geilinger, H. Untersuchungen über das Vorkommen von Jod in der Natur. IX. Über Jodabspaltung und Jodspeicherung durch Mikroorganismen. Biochem. Zeit. **152**:185-190. 1924.
22. Goldschmidt, Viktor. Vererbungsversuche mit den biologischen Arten des Antherenbrandes (*Ustilago violaceae* Pers.) Ein Beitrag zur Frage der parasitären Spezialisierung. Ztschr. Bot. **21**:1-90. 1928.
23. Hanna, W. F. A simple apparatus for isolating single spores. Phytopath. **181**:1017-1021. 1928.
24. ———. Studies in the physiology and cytology of *Ustilago zeae* and *Sorosporium reilianum*. Phytopath. **19**:415-442. 1929.
25. Harter, L. L., and Weimer, J. L. Some physiologic variations in strains of *Rhizopus nigricans*. Jour. Agr. Res. **26**:363-371. 1923.
26. Hayes, H. K., Stakman, E. C., Griffee, Fred, and Christensen, J. J. Reaction of selfed lines of maize to *Ustilago zeae*. Phytopath. **14**:268-280. 1924.
27. Hursh, C. R. The relation of temperature and hydrogen-ion concentration to urediniospore germination of biologic forms of stem rust of wheat. Phytopath. **12**:353-361. 1922.
28. Johnson, T. Studies on the pathogenicity and physiology of *Helminthosporium gramineum* Rab. Phytopath. **15**:363-371. 1925.
29. Jollos, Victor. Experimentelle Vererbungsstudien an Infusorien. Ztschr. Indukt. Abstamm. u. Vererbungslehre. **24**:77-97. 1920.
30. Jones, D. F. Selection in self-fertilized lines as the basis for corn improvement. Jour. Amer. Soc. Agron. **12**:77-100. 1920.
31. Kniep, H. Untersuchungen über den Antherenbrand (*Ustilago violacea* Pers.). Ein Beitrag zum Sexualitätsproblem. Ztschr. Bot. **11**:275-284. 1919.
32. ———. Über morphologische und physiologische Geschlechtsdifferenzierung. Verhrhandl. Physik.-Med. Gesellschaft zu Würzburg., N. F. **46**:1-18. 1921.
33. ———. Über erbliche Änderung von Geschlechtsfaktoren bei Pilzen. Ztschr. Indukt. Abstamm. u. Vererbungslehre **31**:170 183. 1923.
34. ———. Die Sexualität der niederen Pflanzen. Jena, 1928.
35. La Rue, C. D. The results of selection within pure lines of *Pestalozzia guepini* Desm. Genetics **7**:142-201. 1922.
36. Leonian, Leon H. The morphology and the pathogenicity of some Phytophthora mutations. Phytopath. **16**:727-731. 1926.
37. Letcher, Houston, and Willaman, J. J. Biochemistry of plant diseases VIII. Alcoholic fermentation of *Fusarium lini*. Phytopath. **16**:941-949. 1926.
38. Levine, Max, and Carpenter, D. C. Gelatin liquefaction by bacteria. Jour. Bact. **8**:297-306. 1923.

39. Mitter, Julian H. Studies in the genus Fusarium. Saltation in the section discolor. Ann. Bot. **43**:379-409. 1929.
40. Oshima, Kohichi, and Church, M. B. Industrial mold enzymes. Jour. Ind. and Eng. Chem. **15**:67-72. 1923.
41. Ramsbottom, J. The taxonomy of fungi. Trans. Brit. Myc. Soc. **11**:25-45. 1925.
42. Rodenhiser, H. A. Physiologic specialization in some cereal smuts. Phytopath. **18**:955-1003. 1928.
43. Stakman, E. C. Racial specialization in plant disease fungi. Plant Pathology and Physiology in Relation to Man. (Mayo Foundation Lectures, 1926-1927.) W. B. Saunders Co., Philadelphia. 1928.
44. ———, and Christensen, J. J. Heterothallism in *Ustilago zeae*. Phytopath. **17**:827-834. 1927.
45. ———, ———, and Hanna, F. W. Mutation in *Ustilago zeae* (Abst.). Phytopath. **19**:106. 1929.
46. Stevens, F. L. The Helminthosporium foot-rot of wheat, with observations on the morphology of *Helminthosporium* and the occurrence of saltation in the genus. Ill. Dept. of Reg. and Educ., Div. Nat. Hist. Surv. Bull. **14**:77-185. 1922.
47. Zillig, Hermann. Über spezialisierte Formen beim Antherenbrand, *Ustilago violacea* (Pers.) Fuck. Centralbl. Bakt. II. **53**:33-74. 1921.

PLATES

PLATE I

How Mutants Arise

A. Minn. A, a monosporidial line on potato-dextrose-malt agar. Mutants mostly appearing as wedge-shaped or fan-shaped sectors. One patch mutant near the upper left of the colony. Note difference in size, color, and type of growth of the different mutants. It is noteworthy that some of the mutants are much darker in color than the parent colony, while others are much lighter.

B. W. Va. A8-5-1, a mutant derived from W. Va. A8, on potato-dextrose agar. In addition to the varied assortment of fan-shaped mutants, there are patch mutants near the center of the colony.

Minn. A
W. Va. A 8-5-1

PLATE II

Cultural Differences between Mutants and Their Parent

Minn. A and seven of its mutant lines on potato-dextrose-sucrose-malt agar. Note the range in color, topography, and marginal characters among the mutants. Some of the mutants are decidedly darker in color than the parent. Note also the counter-clockwise growth near the margin of Minn. A7.

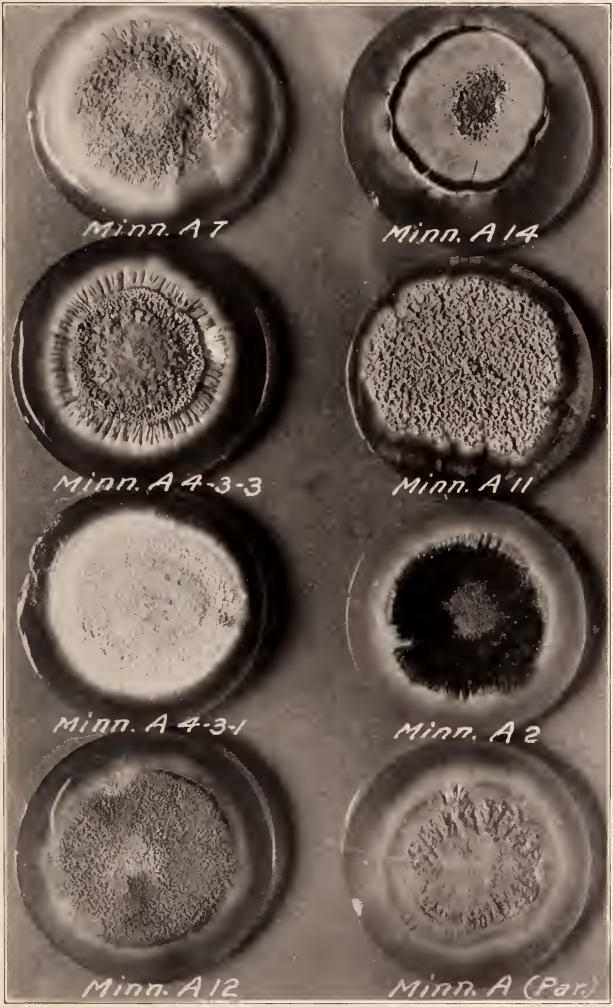

Minn. A 7
Minn. A 14
Minn. A 4-3-3
Minn. A 11
Minn. A 4-3-1
Minn. A 2
Minn. A 12
Minn. A (Par.)

PLATE III

Cultural differences between three mutants from Minn. A on potato-dextrose-sucrose agar. Note counter-clockwise direction of growth in A3-1-1-1. The differences between these three mutants are typical of those between the 70 mutant derivatives obtained from Minn. A.

Minn. A 4-4-4
Minn. A 3-1-1-1
Minn. A 14-4-1

PLATE IV

Eight mutants derived from W. Va. A8, grown under uniform conditions. Note the range of color, topography, zonation, and type of growth. All of the mutants had appeared a year or more previous to the time the photograph was taken, and had retained their distinctive characters on many kinds of media and under many different sets of environmental conditions. Note the decided differences between mutant lines of A8-5, which produced so large a number of the mutants whose origin is shown in Figure 1.

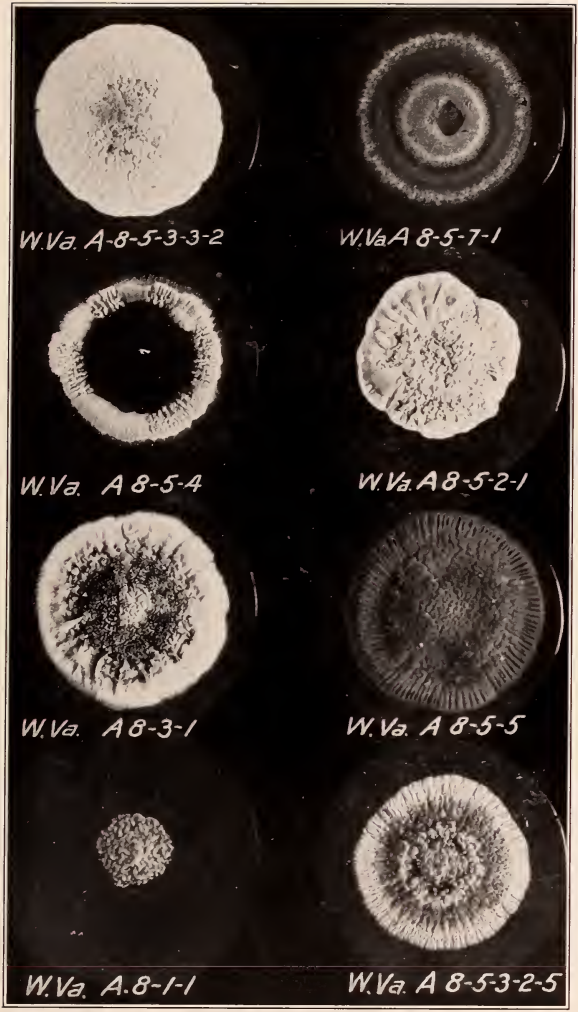

W.Va. A-8-5-3-3-2
W.Va A 8-5-7-1
W.Va. A 8-5-4
W.Va. A 8-5-2-1
W.Va. A 8-3-1
W.Va. A 8-5-5
W.Va. A.8-1-1
W.Va. A 8-5-3-2-5

PLATE V

The differential ability of mutant lines of W. Va. A8 to digest casein, as indicated by clear zone around colonies growing on milk agar. Relative amount of digestion indicated by white area. The photograph was taken through the bottom of the flask; therefore the white medium looks dark in the photograph and the cleared area appears white.

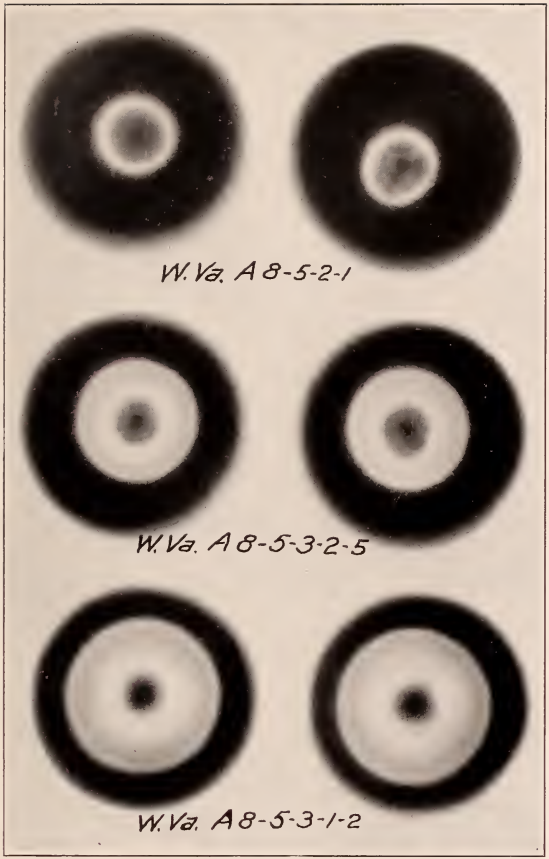

W. Va. A 8-5-2-1
W. Va. A 8-5-3-2-5
W. Va. A 8-5-3-1-2

PLATE VI

Mutants W. Va. A8-3-1, A8-1-1, and A8-5-3-1-2, photographed 24 hours after adding iodine to medium containing KNO_3. Iodine added 13 days after inoculation. It is evident that mutants differ decidedly in rate of action on iodine, indicating that they differ greatly in physiologic characters.

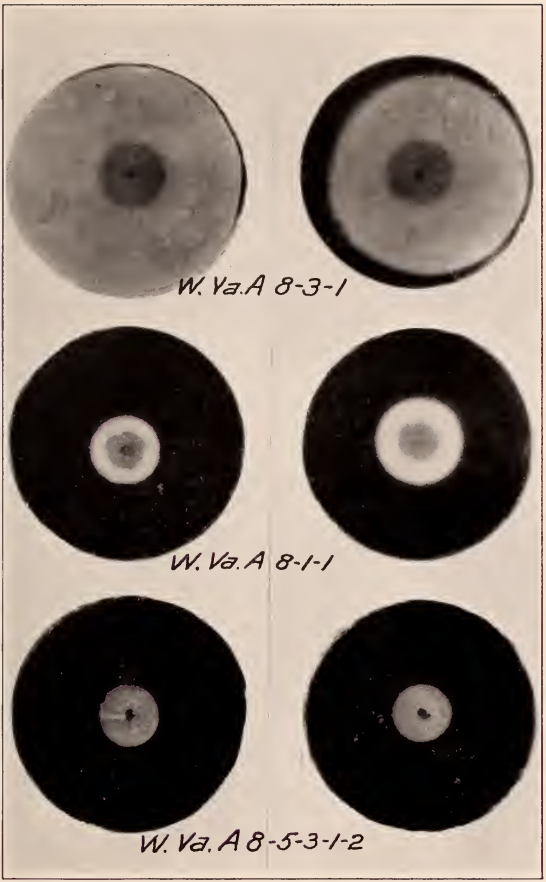

W. Va. A 8-3-1
W. Va. A 8-1-1
W. Va. A 8-5-3-1-2

PLATE VII

Effect of Temperature on Rate of Growth of Different Mutant Lines

Note the difference in ability of lines to grow at the extremes. A8-5-2-1 grows fairly well at 10° and 34° C., whereas A8-5-4 grows scarcely at all at the extremes, and A8-3-1 is intermediate between the two.

PLATE VII

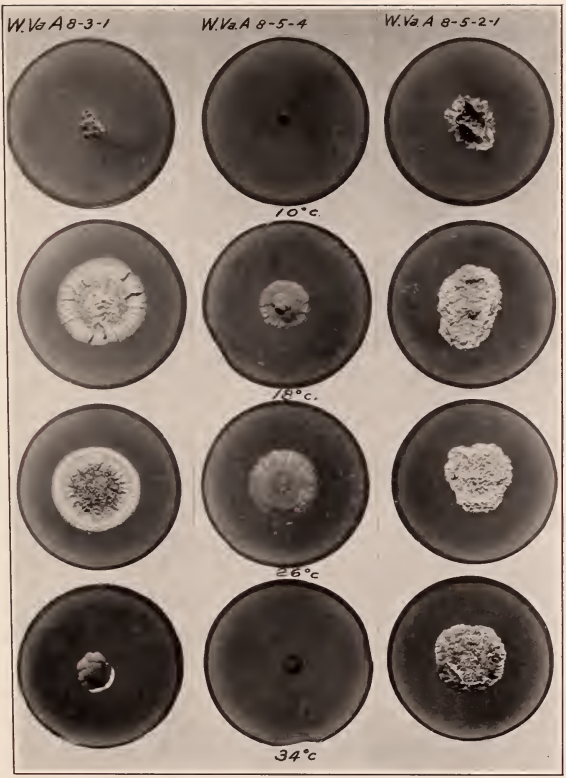


PLATE VIII

Effect of Medium on the Appearance of Colonies of a Single Mutant Line, Minn. A3-1-2

P. A. = potato-extract agar; P. D. A. = potato-extract agar + 3 per cent dextrose; P. A. + 1 per cent glycerine = potato-extract agar + 1 per cent glycerine.

While the appearance of the colonies on the different media is entirely different, it is evident that certain characters tend to remain constant. Certain points of resemblance are apparent on close examination of the colonies, especially those on potato-glycerine agar and potato agar. In general, there are wide variations in the appearance of a single monosporidial line grown on different media.

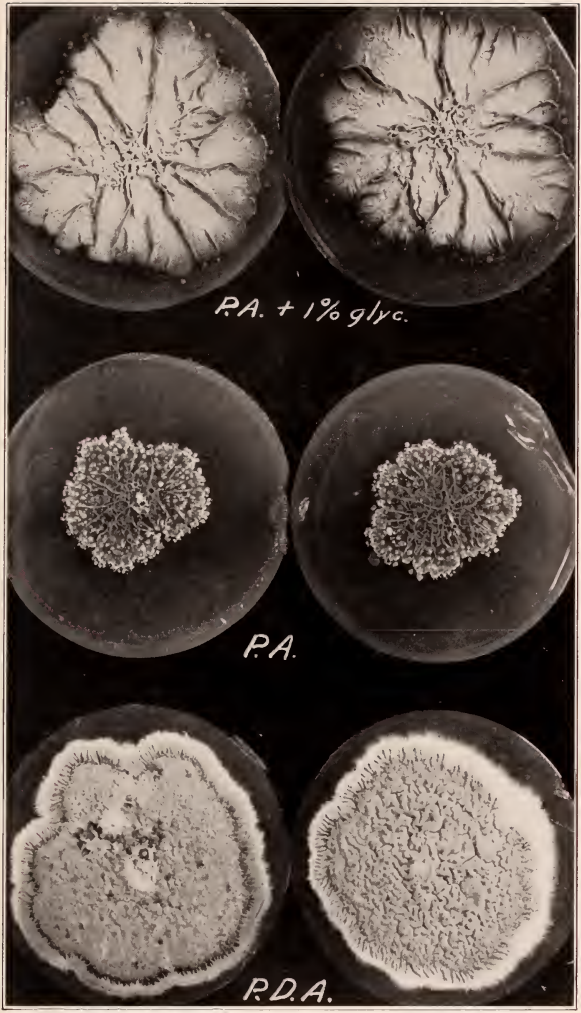

P.A. + 1% glyc.
P.A.
P.D.A.

PLATE IX

Effect of Temperature on the Appearance of Colonies of Mutant Lines

Four mutant lines of W. Va. A8 grown at 18° and 26° C. The mutant in upper left-hand corner was photographed at 10° C. in order to show more clearly the cuplike structure in the center, but unfortunately the photograph did not bring it out.

Note the absence of zonation in W. Va. A8-5-4 at 18° C., also the difference in color from that of the colony grown at 26°. Note also the difference in topography and zonation of the two colonies of W. Va. A8-5-7-1 at the two temperatures.

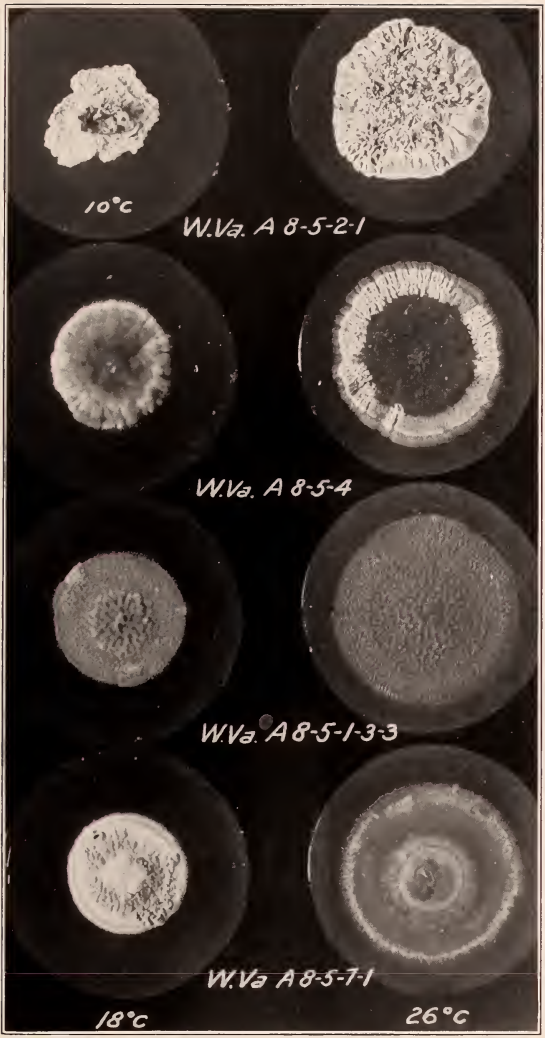
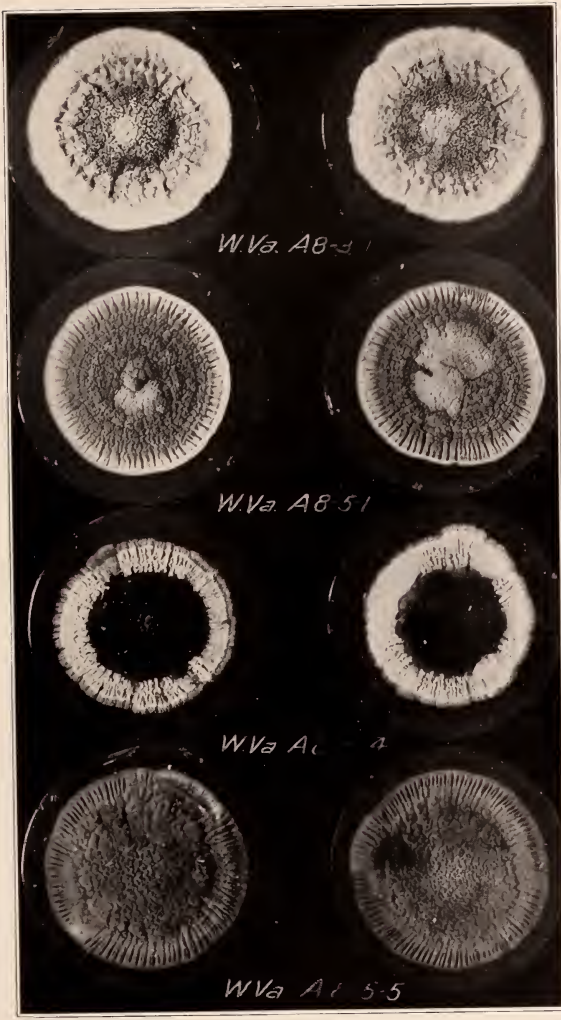

10°C
W.Va. A 8-5-2-1
W.Va. A 8-5-4
W.Va. A 8-5-1-3-3
W.Va A 8-5-7-1
18°C
26°C

PLATE X

Persistence of Cultural Characters

Duplicate colonies of four mutant lines of W. Va. A8 showing cultural characters that have persisted since the lines were isolated; the radial folds and color zones of A8-3-1; the characteristic topography and radial furrows near the margin of A8-5-1; the decided topographic and color zones of A8-5-4. Note the similarity of A8-5-1 and A8-5-5, but note also the slight but distinct differences. The permanence of cultural characters such as these is one of the facts that convince the writers that what they have called mutants are the result of genotypic changes.

PLATE X



Part II. Hybridization

J. J. Christensen[1]

INTRODUCTION

Stakman and Christensen (10), Hanna (6), and Eddins (3) have shown that *Ustilago zeae*, like most other smut fungi investigated (8, 9), is usually heterothallic. This means that the fusion of two monosporidial lines of opposite sex usually is prerequisite to normal infection and production of chlamydospores in the smut galls on corn plants. The sporidia are haplonts; they are potential gametes. Monosporidial, that is, gametic, lines propagate by budding, by growth of haploid mycelium, or by both methods—in nature and in nutrient media; hence it is possible to study the characters of gametic lines in culture. The results of such studies are reported in Part I, where it is shown that gametic lines may differ from each other in many characters other than sex and that numerous new lines arise by mutation. It is desirable to know, also, to what extent new lines arise as a result of hybridization. For this reason crosses were made between monosporidial lines and a study was made of lines derived from the sporidia produced on the promycelia of the resulting chlamydospores.

ARE THERE "GEOGRAPHIC STRAINS"?

Hanna (6) showed that promycelia of *U. zeae* might be either bisexual or quadrisexual, i.e., the 4 primary sporidia might belong to 2 or to 4 sexual groups. In this respect *U. zeae* is similar to many of the gill fungi, in which the 4 basidiospores on a basidium may belong either to 2 or to 4 sexual groups. But Kniep (7), Hanna (5), and Vandendries (11) showed that there are "geographical strains" in *Schizophyllum commune, Coprinus lagopus,* and *C. micaceus,* respectively. For example, according to Hanna (5), the basidiospores of any individual fruiting body of *C. lagopus* belong to 4 sexual groups only, and the mycelia from these 4 spores also would belong to 4 sexual groups. When, however, pairings were made between 11 mycelia derived from one wild fruiting body and 11 from another, there was complete fertility between them. In 6 fruiting bodies studied in this manner there were 24 distinct sexual groups instead of the 4 which might be expected. Kniep already had shown that, while the spores of individual fruiting bodies of *Schizophyllum commune* belong to 4 sexual groups, there was complete interfertility between different mycelia of monosporous origin derived from fruiting bodies collected at some distance from each other. Vandendries (11) obtained similar results with two wild fruiting bodies

[1] The writer is indebted to Dr. E. C. Stakman for help in the preparation and arrangement of material.

of *Panaeolus campanulatus*. He found, on the other hand (12), a high degree of intersterility between some strains of *Coprinus micaceus*.

Stakman and Christensen (10) showed in the case of *U. zeae* that normal infection might result from inoculating corn with paired lines of different sex from the same locality and also from widely separated geographic regions. For example, monosporidial lines from New Hampshire, Mississippi, and Canada produced normal galls when paired with monosporidial lines from Minnesota, and different lines from Minnesota also paired with each other. Later, Hanna (6) successfully crossed monosporidial lines from Italy with some from Minnesota, and showed that the sexual groups of the Minnesota collection apparently were identical with those of the Italian collection.

During 1928 and 1929 further experiments of this nature were made. Minn. E and Minn. F, two lines of opposite sex isolated from material collected at University Farm, St. Paul, Minnesota, were paired with 18 monosporidial lines, all culturally different, obtained from several different countries. Results are summarized in Table I. It will be seen that all of the lines paired successfully with either Minn. E. or Minn. F. There were differences in the virulence of the various combinations, but the results are not recorded here. It is evident, however, that lines from widely separated regions and those from the same region may or may not hybridize. There is no evidence either of complete interfertility or intersterility. Each line tried, regardless of its origin, united either with Minn. E or with Minn. F but not with both.

TABLE I

Results of Inoculating Golden Bantam Corn with Monosporidial Lines of *Ustilago zeae* from Different Geographic Regions in Combination with Minn. E and Minn. F, Two Lines of Opposite Sex

Lines	Lines and gall formation*	
	Minn. E	Minn. F
Colorado A	+	—
West Virginia A7	+	—
West Virginia A8	+	—
Canada A	+	—
France A	+	—
Hungary A	+	—
Italy B	+	—
Spain B	+	—
Minn. F	—	+
Minn. A	—	+
Pennsylvania A	—	+
Texas A	—	+
Texas B	—	+
West Virginia A	—	+
France B	—	+
Italy A	—	+
Italy C	—	+
Spain A	—	+

* + = gall formation; — = none.

TERMINOLOGY

Monosporidial lines of *U. zeae* are characteristically haploid. When two lines of opposite sex are grown together on artificial media their mycelia or sporidia may intermingle, but apparently do not fuse (6, 10). When, however, they are inoculated into corn plants, haploid hyphae of opposite sex fuse, the nucleus from each haploid hypha passing into the fusion cell, which then would contain two nuclei of opposite sex. These nuclei do not fuse at once but remain associated and divide conjugately, so that each cell of the resulting mycelium, the dikaryophase, is binucleate. Plasmogamy has therefore occurred, but karyogamy is delayed until the chlamydospores are formed, when the two nuclei of each spore-mother cell fuse, thus giving rise to the true diplophase. The chlamydospore nucleus is a fusion nucleus; therefore the spores are zygotes. On germination the chlamydospores produce a promycelium, the fusion nucleus divides, the two daughter nuclei in turn divide, thus resulting in the formation of four nuclei, one for each cell of the typically four-celled promycelium. A primary sporidium is then formed on each cell of the promycelium, each nucleus again divides, one from each cell passing into the sporidium, and one remaining in the promycelial cell. As reduction division occurs in the promycelium (6), the sporidia are haplonts. They are functional gametes that can propagate asexually, making it possible to study the characters of gametic lines.

When crosses are made between gametic lines of opposite sex, then, the immediate result is the dikaryophase, followed by the true diplophase, which in turn gives rise to the haploid phase. How are these phases to be interpreted genetically? Clearly the sporidia or mycelia of monosporidial lines are, at least partly, gametic in nature. Possibly in the strictest sense the gametes are the haploid nuclei, altho the sporidia may equally well be considered as gametes. But what about the dikaryophase; is it unique in the fungi, or is it approximately equivalent to part of the F_1 generation in higher plants? The answer depends on one's interpretation of the essential criteria of the diploid condition. Some mycologists consider that the dikaryophase is essentially equivalent to the diplophase because of the well-established fact that the two nuclei clearly influence the morphology, growth, and pathogenicity of the hyphae. Haploid hyphae, containing nuclei of one sex only, are usually rather fine, in the host plant, and normally cannot grow extensively in the tissues. When nuclei of opposite sex are associated in the hyphae, however, the hyphae are large, often grow vigorously, and are distinctly pathogenic. Consequently there seems to be some justification for the argument that conjugate nuclei exert approximately the same sort of effect on the hyphae as do fusion nuclei. If it is agreed

that there is no essential difference between the dikaryophase and the diplophase, the first fusion cell is the zygote and the F_1 generation begins with the growth of the mycelium from that cell. The F_1 would then include the mycelium in the corn tissues, the chlamydospore, and the promycelium, where gametogenesis—the production of sporidia, or, if one prefers, of the haploid nuclei—occurs. If the essential identity of the dikaryophase and true diplophase is denied, the chlamydospores are zygotes, the F_1 is restricted to the promycelium, and the dikaryophase has no real counterpart in higher plants. In either case the sporidia are the F_1 gametes, unless the haploid nuclei, only, are considered as gametes. Cultures derived from single sporidia following a cross are designated in this paper as F_1 gametic lines (or merely as f_1 lines), for they really are gametic lines whether the entire sporidium or its nucleus, only, is considered as the true gamete. For convenience, the monosporidial lines used in making the crosses are designated parents or parental lines (p_1). In reality they are the parental gametes, but it may be permissible to refer to them as parents because it simplifies the terminology.

CROSSES MADE FOR STUDY OF f_1 LINES

The following crosses were made: Minn. A13 x Minn. F; Minn. A13 x Minn. D; Minn. A13 x Canada A; Minn. A x Canada A; Minn. A12 x Minn. D; and Minn. A x Minn. F. Minn. A, Minn. D, and Minn. F originated from material collected at University Farm, St. Paul, Minnesota, Minn. A and Minn. D having been isolated from the same promycelium. Minn. A12 and Minn. A13 are sister mutants from Minn. A. Canada A was derived from a smut gall obtained from Canada. For convenience, the sporidia were numbered in succession as they were obtained from germinating chlamydospores resulting from a given cross. Thus, Minn. D x Minn. A13.2 refers to the second sporidium isolated from cross Minn. D x Minn. A13. The crosses were made by mixing liquid cultures of the monosporidial lines and injecting the mixture into the corn plants with a hypodermic syringe. The method was essentially that described by Stakman and Christensen (10).

The sporidia were not taken directly from the promycelia. Instead, the chlamydospores were sown on agar media and, after they had germinated and produced sporidia, isolations were made from various places in order to obtain sporidia from as many different chlamydospores as possible. In all, 102 individual sporidia were isolated from the 6 crosses. The individual sporidia were first placed in Van Tieghem cells and, after they had multiplied sufficiently, transfers were made to agar slants and allowed to develop for 3 or 4 days. Transfers were

then made to a series of triplicate flasks containing 30 cc. of Difco potato-dextrose agar. These cultures then were used for comparative tests. The crosses are indicated in the usual manner and the f_1 (monosporidial) lines from each cross are numbered serially. Thus, Minn. A13 x Minn. F.6 indicates the monosporidial line obtained from the sixth sporidium isolated from germinating chlamydospores resulting from the cross Minn. A13 x Minn. F. When single sporidia were isolated from a monosporidial line such as Minn. A13 x Minn. F.6, the lines were designated in the same manner. Thus, Minn. A13 x Minn. F.6.1 indicates the first monosporidial line isolated from the sixth monosporidial line from cross Minn. A13 x Minn. F.

CHARACTERS OF f_1 LINES

Sexual Characters[2]

Heretofore there has been evidence of four sex groups in *U. zeae*. Stakman and Christensen (10) found only two, while Hanna (6) found that the four sporidia on certain promycelia fell into two sexual groups, and those on others belonged to four groups. In the first case the results indicated that difference in sex was due to a single factor difference while in the second, at least two factor pairs were involved. In the present work the author made a large number of crosses in order to determine the pathogenic capabilities of different combinations of lines. As pathogenicity depends so largely on sexual reactions between different lines, an attempt was made to find out how many sexual groups there were among the F_1 gametes from crosses. The results are given in Tables II, III, and IV.

In Table II are recorded the results of pairing 13 f_1 lines from Minn. A13 x Minn. F with the parents and with 3 other monosporidial lines. There are at least 5 sexual groups among the 13 f_1 lines, on the basis of their ability to form chlamydospores when paired with other lines. Lines 5, 8, 7, and 10 appear to be identical sexually with Minn. A13, while line 2 is like Minn. F. Lines 3 and 13 did not unite with either parent, but each united with one of the testers, and lines 1, 4, 6, 9, 11, and 12 failed to unite with any of the lines with which they were mated.[3] Minn. F and A13 x F.2 both formed a virulent combination with Minn. A, but were less virulent with its mutant A13, while

[2] The writer has used as a criterion of sex differences the ability of lines to produce chlamydospores in the host plant. If two lines do not produce chlamydospores, they are considered to be of the same sex. It is conceivable, of course, that such lines may produce dikaryophytes which cannot grow in the corn tissues because of the absence of necessary factors for pathogenicity. There is strong evidence, however, including microscopic studies, indicating that this is not true.

[3] It was found subsequently that lines 1 and 6 both united with line 9, but 1 and 6 did not unite; hence 1 and 6 appear to belong to one sexual group and 9 to another. In the discussion of Table II, however, conclusions are based only on data available when the table was prepared.

A13 x F.3 united readily with A, but not at all with A13. It will be noted also that lines 5 and 8, like A13, produced only light infection with Minn. F, but 7 and 10 caused heavy infection with this line. It is a question whether the differences in degree of infection are due to sex factors or to distinct factors for pathogenicity. If they are due to the former, there are six sexual groups instead of five, but, for reasons given later, the author has used as a criterion of sex differences only the ability to form chlamydospores, altho recognizing the possibility that degree of infection also may be an indication of them.

TABLE II

Results of Inoculating Golden Bantam and Minn. 13 Corn in the Greenhouse with f_1 Lines from Minn. A13 x Minn. F. in Combination with the Parents and with Minn. A, Minn. B, and Minn. D.

Parents and f_1 lines	Lines and infection					Lines and degree of infection*				
	A	A13	F	B	D	A	A13	F	B	D
A13	—	—	+	+	+	—	—	L	H	H
A13 x F.5	—	—	+	+	+	—	—	L	H	H
do 8	—	—	+	+	+	—	—	L	H	H
do 7	—	—	+	+	+	—	—	H	H	H
do 10	—	—	+	+	+	—	—	H	H	H
F	+	+	—	—	—	H	L	—	—	—
A13 x F.2	+	+	—	—	—	H	L	—	—	—
do 3	+	+	—	—	—	H	—	—	—	—
do 13	—	—	—	—	+	—	—	—	—	VL
do 1	—	—	—	—	—	—	—	—	—	—
do 4	—	—	—	—	—	—	—	—	—	—
do 6	—	—	—	—	—	—	—	—	—	—
do 9	—	—	—	—	—	—	—	—	—	—
do 11	—	—	—	—	—	—	—	—	—	—
do 12	—	—	—	—	—	—	—	—	—	—

* + = infection; — = no infection; H = heavy infection; L = light infection; VL = very light infection.

Table III summarizes the results of pairing thirteen f_1 lines from Minn. A12 x Minn. D with the parents and, to some extent, with each other. To the right of the table the lines are grouped according to their sexual reactions with the parents. Lines 4, 8, 10, and 13 are like A12 in that they combine with D and not with A12; lines 5, 6, 9, and 12 are like D, as they combine with A12 but not with D; and 1, 2, 3, 7, and 11 fail to combine with either parent. With respect to their reactions with the two parents, therefore, there appear to be only three sexual groups. But an examination of the complete table shows that there are at least nine groups. Only 8 and 13 are like A12, and 5 only is like D. Lines 1, 2, 3, 7, and 11 appear identical when mated with the parents only, but, on the basis of their reactions with other lines, it is clear that they are different, with the possible exception of 3, which was dropped from consideration because it was mated with the parents only. There apparently are at least nine sexual groups on the basis of

the pairings that were made. In addition to those lines that appear to be identical with the parents, 4 and 10 seem to be alike, and 6 and 9 are alike on the basis of the matings made. Line 12 is like 6, but not like 9, which is assumed to be identical with 6. Whichever combination is assumed, the total number of groups is not changed. All possible combinations were not made; had they been, it is possible that still more sexual groups could have been demonstrated.

TABLE III

RESULTS OF INOCULATING GOLDEN BANTAM AND MINN. 13 CORN WITH f_1 LINES FROM MINN. A12 x MINN. D WITH THE PARENTS AND WITH EACH OTHER

Lines		Lines and infection*																		
		A12 x D.																		
		A12	D	1	2	3	4	5	6	7	8	9	10	11	12	13	Group	A12†	D	
Parents	A12	o	+	–	–	–	–	+	+	–	–	+	–	–	+	–	1	A12	–	+
	D	+	–	–	–	–	+	–	–	–	+	–	+	–	–	+	2	4	–	+
A12xD.	1	–	–	o	–	o	–	–	–	+	o	–	o	–	o	o	3	8	–	+
	2	–	–	–	o	o	+	–	–	–	o	–	+	+	o	o	4	10	–	+
	3	–	–	o	o	o	o	o	o	o	o	o	o	o	o	o	..	13	–	+
	4	–	+	–	+	o	o	+	o	–	–	+	–	–	–	o	5	D	+	–
	5	+	–	–	–	o	+	o	o	–	+	–	+	–	–	o	2	5	+	–
	6	+	–	–	–	o	o	o	o	o	o	–	o	+	o	o	6	6	+	–
	7	–	–	+	–	o	–	–	o	o	o	–	o	o	–	o	7	9	+	–
	8	–	+	o	o	o	–	+	o	o	o	+	o	–	+	o	1	12	+	–
	9	+	–	–	–	o	+	–	–	–	+	–	+	+	–	+	6	1	–	–
	10	–	+	o	+	o	–	+	o	o	o	+	o	–	–	o	5	2	–	–
	11	–	–	–	+	o	–	–	+	o	–	+	–	o	+	–	8	3	–	–
	12	+	–	o	o	o	–	–	o	–	+	–	–	+	o	+	9	7	–	–
	13	–	+	o	o	o	o	o	o	o	o	+	o	–	+	o	1	11	–	–

* + = infection; – = no infection: o = no test.

† Lines grouped according to reaction with parents.

Here again, as in Table II, only the production of chlamydospores is considered in determining the number of sexual groups. If the degree of infection also were considered, lines 8 and 13 would be classed as different, as 13 formed less virulent combinations with Minn. D and with A12 x D.12 than did 8.

During the winter of 1928-29 inoculations were made in the greenhouse with lines from Minn. A13 x Minn. D. The results indicated that there were many sexual groups, and that some lines caused infection when inoculated singly into corn plants. Consequently, it was deemed advisable to make a more extensive test in the field. In the summer of 1929, therefore, the combinations indicated in Table IV were made. Thirty-one f_1 lines from Minn. A13 x Minn. D were paired with the parents; with certain other f_1 lines from the same cross; with Minn. F; with two f_1 lines from Minn. A13 x Minn. F; and with Minn. A6-1, which, like Minn. A13 is a mutant of Minn. A. Certain combinations also were made between the testers. In addition, each line was inoculated singly into corn plants. For each combination

25 to 40 plants, each, of normal Golden Bantam and Gehu corn, 8 to 12 inches tall, were inoculated. Ample checks, which remained uninfected, were injected with sterile nutrient solution. In addition, the inoculations with unpaired lines actually served as checks. The results are summarized in Table IV.

TABLE IV

RESULTS OF INOCULATING GOLDEN BANTAM AND GEHU CORN IN THE FIELD WITH f_1 LINES FROM MINN. A13 x MINN. D, WITH SIMILAR LINES FROM MINN. A13 x MINN. F, AND WITH CERTAIN OTHER LINES OF *Ustilago zeae*, IN COMBINATIONS WITH ONE ANOTHER

f_1 lines		Lines and infection*																	
		Minn. A13 x Minn. D.										A13 x F.6	A13 x F.9	Minn. A6-1	Minn. A13	Minn. D	Minn. F	o†	Group‡
		1	4	14	17	19	22	25	30	31									
Minn. A13	32	—	—	—	—	—	—	—	—	—		—	—	—	—	—	—	—	1
x	31	—	—	—	—	—	—	—	+	—		—	—	—	—	—	—	—	2
Minn. D.	30	—	—	—	—	—	—	—	—	+		—	—	—	—	—	—	—	3
	4	—	—	—	—	—	+	—	—	—		—	—	—	—	—	—	—	4
	16	—	—	—	—	—	+	—	—	—		—	—	—	—	—	—	—	
	14	—	—	—	—	—	—	—	—	—		—	—	+	—	—	—	—	5
	19	—	—	—	—	—	—	—	—	—		—	—	—	—	—	+	—	6
	26	—	—	—	—	—	—	—	—	—		—	—	—	—	—	+	—	
	1	—	—	—	—	—	+	—	—	—		—	+	—	—	—	—	—	7
	2	—	—	—	—	—	+	—	—	—		—	+	—	—	—	—	—	
	5	—	—	—	—	—	+	—	—	—		—	+	—	—	—	—	—	
	7	—	—	—	—	—	+	—	—	—		—	+	—	—	—	—	—	
	21	—	—	—	—	—	+	—	—	—		—	+	—	—	—	—	—	
	28	—	—	—	—	o	+	—	—	—		—	+	—	—	—	—	—	
	3	—	—	—	—	+	—	—	—	—		—	—	+	—	—	—	—	8
	17	—	—	—	—	—	—	—	—	—		—	—	—	—	—	+	+	9
	18	—	—	—	—	—	—	—	—	—		—	—	—	—	—	+	+	
	12	+	+	—	—	—	—	—	—	—		+	—	—	—	—	—	—	10
	13	—	+	—	—	—	—	+	—	—		+	—	—	—	—	—	—	11
	23	—	—	—	—	—	+	—	—	+		—	+	—	—	—	—	—	12
	24	—	—	—	—	—	+	—	—	—		—	+	+	—	—	—	—	13
	25	—	—	—	—	—	+	—	—	—		—	+	+	—	—	—	—	
	20	+	+	—	—	—	—	+	—	—		+	—	—	—	—	—	—	14
	22	+	+	—	—	—	—	+	—	—		+	—	—	—	—	—	—	
	11	—	—	—	—	+	+	+	+	—		—	—	—	+	—	+	—	15
	15	+	+	—	—	—	+	+	+	—		+	—	—	—	—	—	—	16
	27	+	+	—	—	—	+	+	—	—		+	—	—	—	—	+	—	17
	8	+	+	+	—	—	—	+	—	—		—	—	—	—	+	+	—	18
	6	+	+	+	+	+	+	+	+	+		o	+	o	+	+	+	+	19
	9	+	+	o	o	+	+	+	+	+		o	o	+	+	o	+	+	
	10	+	+	+	+	+	+	o	o	+		+	+	o	+	o	+	+	
A13 x F.6		—	—	—	—	—	+	—	—	—		—	+	o	—	—	—	—	7
A13 x F.9		+	—	—	—	—	—	+	—	—		+	—	—	—	—	—	—	20
Minn. A6-1		—	—	+	—	—	+	—	+	+		o	—	—	—	+	+	—	21
Minn. A13		—	—	—	—	—	—	—	—	—		—	—	—	—	+	+	—	22
Minn. D		—	—	—	+	—	—	—	—	—		—	—	+	—	—	—	—	23
Minn. F		—	—	—	+	+	—	—	—	—		—	+	+	—	—	—	—	24

* + = infection; — = no infection; o = no test.

† Results of inoculations with unpaired lines.

‡ Based on reactions indicated in horizontal columns only.

It will be seen from Table IV that the 31 f_1 lines from A13 x D, listed vertically, fall into 19 groups on the basis of their reactions with the lines listed horizontally. Two lines, 17 and 18, appear to be identical with A13; line 18 may be, but 17 is not because A13 combines with 11, and 17 does not (compare vertical columns in the table). None of the lines are like Minn. D, the other parent. The groupings, indicated in Table IV, do not necessarily bring together identical lines, as shown by the apparent but not real identity of 17 and A13 when the horizontal columns only are considered. Again, lines 4 and 16 combined with only one of the lines listed horizontally and appear to be the same, but in reading the table vertically it is evident that line 4 combines with lines 8, 12, 13, 15, 20, 22, and 27. As line 16 was not paired with all of the lines listed vertically, there is no way of knowing whether it belongs to the same group as 4. Had all possible combinations been made, additional sexual groups probably would have become evident. In any case, the minimum number of sexual groups resulting from A13 x D is 19, including the group that cause infection singly. There appear, therefore, to be multiple factors for sex.

A13 x D.6, 9, and 10 caused infection when injected singly into corn plants. As the degree of infection was no weaker when these lines were used alone than when they were combined with other lines, it seems likely that they did not unite with them sexually. It is not known whether these lines satisfy the criteria for true homothallism, i.e., bisexuality. They may complete their life cycle in the haplophase; or possibly the nucleus in the sporidia of these lines is diploid; the fusion nucleus in the chlamydospores may not have undergone reduction division. If so, the lines are merely parthenogenetic. A study of their cytology is now under way.

There were decided differences in the degree of infection caused by the different combinations of lines. All combinations with line 11 were very weak pathogenically, incipient galls only being produced with lines 22 and 25, very light infection resulting with Minn. F and lines 19 and 30, and light infection with Minn. A13. Line 1 caused heavy infection in combination with six lines, and very light infection with one. Again, line 27 caused heavy infection in combination with three lines and very light infection with three others. The results indicate that there are multiple factors for pathogenicity as well as for sex.

Further Experiments with Lines That Caused Infection Singly

As it appeared from the previous experiments that Minn. A13 x Minn. D.6, 9, and 10 could cause infection singly, another field test was made in August, 1929. The results are given in Table V. A high percentage of infection resulted from inoculations with these monosporidial lines, as indicated by marked coloration on the leaves. More

than 50 per cent of the plants inoculated with A13 x D.6 developed normal smut galls. The plants were rather small at the time of inoculation, and it was dry and hot for two weeks subsequently. This may possibly account for the relatively low percentage of plants with galls. There was no indication of any contamination. Not a single smut gall developed on plants inoculated with two unisexual lines, Minn. F and Minn. A13 x Minn. F.1. It appears certain from these experiments, therefore, that A13 x D.6, 9, and 10 cause infection singly, and Minn. A12 x Minn. D.6 appears to be decidedly more virulent than the other two

TABLE V

Results of Inoculating Golden Bantam Corn in the Field with 'Homothallic' Lines and with Minn. F, and with Minn. A1 3x Minn. F.1

Lines	No. plants inoculated	No. plants infected	No. plants with galls
Minn. A13 x Minn. D.6	79	77	41
do 9	73	64	11
do 10	83	80	15
Check Minn. A13 x Minn. F 1	66	0*	0
Check—Minn. F	40	0*	0
Check†	55	0	0

* When this line is paired with a line of opposite sex strong infection results.

† Plants inoculated with sterile nutrient solution.

In order to make absolutely certain that the results were not due to mixtures in the cultures, resulting from contamination with lines of another sex, or from sex mutation, several monosporidial isolations were made from each line in the usual manner. These subcultures were tested in the greenhouse, as indicated in Table VI. Many of the Golden Bantam plants inoculated with the sub-monosporidial cultures of Minn. A13 x Minn. D.6, 9, and 10 developed normal smut galls. However, some of the results were erratic. No infection resulted when Golden Bantam plants were inoculated with the following sub-monosporous cultures: A13 x D.9.1, A13 x D.10.4, and A13 x D.10.6. In a preliminary test, however, subculture Minn. A13 x Minn. D.6.2 was found to be virulent, having caused infection and produced large galls on two out of three plants 12 inches high. It is difficult to explain these results; they may have been due to mutation of the organism, which is known to be common in these three lines. It is possible also that the reduction division of the diploid nucleus did not occur in the usual manner in the promycelium, that the primary sporidia were diploid, and that reduction division occurred in some but not in others during the process of budding. Some sporidia would then be diploid and some haploid.

The evidence that lines 6, 9, and 10 cause infection singly seems conclusive. Betwen 500 and 600 plants of Golden Bantam and Gehu

were inoculated with each line, and there was a high percentage of infection. The experiment was repeated, with similar results. Finally, monosporidial cultures again were made from the above monosporidial lines, and when inoculated into corn plants these lines also caused infection, with the exceptions noted. A cytologic study of these lines has not yet been made, but, whatever the nuclear condition, it is certain that some monosporidial lines can alone cause infection, with the production of galls containing chlamydospores. This ability seems to be rare in the Ustilaginales, altho Boss (1) states that *Ustilago ischaemi* completes its life cycle in the haplophase, both in artificial cultures and in nature.

TABLE VI

RESULTS OF INOCULATING GOLDEN BANTAM CORN IN THE GREENHOUSE WITH SUB-MONOSPORIDIAL CULTURES ISOLATED FROM CULTURES OF THREE 'HOMOTHALLIC' LINES

Lines	No. plants inoculated	No. plants infected	No. plants with galls
Minn. A13 x Minn. D.6.1	26	19	11
do .2	22	1	1
do .3	19	16	13
do .4	25	16	9
do .5	19	12	9
Minn. A13 x Minn. D.9.1	16	0	0
do .2	19	2	1
do .3	19	2	2
do .4	20	5	1
do .5	19	8	4
do .6	14	6	2
Minn. A13 x Minn. D.10.1	16	14	8
do .2	22	3	2
do .3	20	10	4
do .4	24	0	0
do .5	15	5	0
do .6	16	0	0
Check-Minn. A13 x Minn. F.7	25	0	0
do .9	9	0	0
Check-Minn.A1	22	0	0

Cultural Characters

All the f_1 lines from the six crosses were studied comparatively on solid media. A general summary of their characteristics is given in Table VII, and a more detailed summary of the character of lines from Minn. A13 x Minn. F are given in Table VIII. Most of the lines of a given cross were strikingly different from their parents and from each other in one or more of the following characters: type and rapidity of growth, color, topography of colonies, production of sporidia and aerial mycelium, and tendency to mutate. Some of the differences between lines are illustrated in Plates II and III. It is interesting to note that the differences between the f_1 lines were as great as those between the lines obtained from many different countries.

TABLE VII

A Comparison of the Characteristics of the f_1 Lines from Six Different Crosses with the Characteristics of the Parental Lines

Crosses	No. F_1 gametes isolated	No. sexual groups	Crosses	Type of growth* of f_1 lines			Cultural characters†			No. f_1 lines mutating	Color‡
				S	SM	M	Similar to parents	Similar to each other	No. different types of colonies		
Minn. A13 x Minn. F	13	At least 6	S x M	7	2	4	0	7 and 8 12 and 13	11	8	1 like Minn. F, 1 like Minn. A13, 9L., 2D
Minn. A13 x Minn. D	32	Many	S x M	11	7	14	0	23 and 24	31	21	4 like Minn. A13, 17L., 3D, 8I
Minn. A13 x Canada A	5	—§	S x M	3	2		0	0	5	5	1 like Minn. A13, 1 like Canada A, 1L., 2D
Minn. A x Canada A	25	—	M x M	21	2	1	—	—	—	15	7 like Minn. A, 8 like Canada A, 10D
Minn. A12 x Minn. D	13	At least 9	M x M	1		12	1 like Minn. A12	0	13	8	2 like Minn. A12, 7L., 4D, 1I
Minn. A x Minn. F	14	—	M x M	1		13	0	5 and 6 12 and 13	12	5	3L., 5D, 6I

* M = mycelial; S = sporidial; SM = semi-mycelial.

† Cultural characters include topography, surface, and margin.

‡ L = lighter than the lighter parent; D = darker than the darker parent; I = intermediate between light and dark parent.

§ — = no test.

None of 77 lines obtained from Minn. A13 x Minn. F, Minn. A13 x Minn. D, Minn. A13 x Canada A, Minn. A12 x Minn. D, and Minn. A x Minn. F were like their parents in cultural characteristics. (See Table VII.) It is unfortunate that cultural characteristics of f_1 lines from Minn. A x Canada A were not recorded. A number of these were quite similar to, if not identical with, each other, and some resembled closely the cultural characters of the parents. Among the f_1 lines from Minn. A13 x Minn. F and from Minn. A x Minn. F there were two sets of duplicates each; from Minn. A13 x Minn. D, one; and none of those from Minn A13 x Canada A and Minn. A13 x Minn. D were alike. Mutants Minn. A12 and Minn. A13 when crossed with the same line, Minn. D, gave rise to f_1 lines which were all different from each other. The results indicate that multiple factors govern most cultural characters.

From the cross Minn. A12 (mycelial) x Minn. D (mycelial) 12 sporidia (F_1 gametes) produced mycelial colonies, and only one a sporidial colony. Minn. A13 (sporidial), a sister mutant to Minn. A12 (mycelial), crossed also with Minn. D (mycelial) gave rise to 14 mycelial, 7 semi-mycelial, and 11 sporidial types. In another cross, Minn. A13 (sporidial) x Minn. F (mycelial), the sporidial types predominated over those of the mycelial type. But Minn. F (mycelial) x Minn. A (mycelial), the parent of Minn. A13, gave rise to 1 sporidial and 13 mycelial f_1 lines. It seems evident from these breeding tests that the mutants Minn. A12 (mycelial) and Minn. A13 (sporidial) are genetically different from each other, and A13 is different from its parent, Minn. A. It is significant that Minn. A crossed with Minn. F and with Minn. D gave rise to different ratios of mycelial to sporidial types. Therefore, Minn. F and Minn. D apparently do not carry the same genetic factors for type of growth.

There apparently are multiple factors for color of colonies. Of the 32 f_1 lines from Minn. A13 x Minn. D, 17 were lighter than either parent; 3 were distinctly darker than the darker parent; 4 were like one parent, Minn. A13, in color; and the other 8 were intermediate in color between the light parent and the dark parent. Twenty-six of the 32 lines produced colonies distinctly different in color from each other. Minn. A12 x Minn. D.7 was conspicuously darker than the darker parent, which was reddish brown. A12 x D.7 was coal black and gave rise almost immediately to several pure white mutants, that have remained white for several generations. There also was one blackish line among the 25 isolated from Canada A x Minn. A. Both parents in this case were cream to tan in color. Seven gametic lines from the same cross resembled one of the parents in color, and 8 the other parent. None were lighter than the parents, but 10 were darker than either parent. Among the lines obtained from all the crosses, with the exception of Canada A x Minn. A, there were many kinds

and gradations of color. The evidence tends to show that multiple factors are concerned in color production. The results indicate also that the mutants, Minn. A12 and Minn. A13, do not possess the same factors for color as their parent Minn. A.

Mutability

Some of the lines derived from six crosses listed in Table VII mutated frequently; others did not mutate during the first cultural generation in flasks. (See Table VIII.) It is obvious that Minn. A13 x Minn. F.1 and Minn. A13 x Minn. F.11 were very unstable. Three colonies of the former gave rise to 16 mutants, 4 of which were different; and the latter line produced more than 20 mutants, 5 of which were distinctly different from the parent and each other. The f_1 lines from Minn. A13 x Canada A were the least stable; they gave rise to the largest number of mutants per colony, while those from Minn. A x Minn. F apparently were the least mutable of the six crosses studied.

TABLE VIII

CULTURAL CHARACTERISTICS OF THIRTEEN f_1 LINES FROM MINN. A13 x MINN. F AND OF THE PARENTAL LINES WHEN GROWN ON NUTRIENT MEDIA

Parents and f_1 lines*	Diam. in mm.	Growth on potato-dextrose agar			Sporidia production in 1 per cent malt + 1 per cent sucrose solution
		Type†	Color‡	No. sectors	
Minn. F	46	M	Lilac	0	Light
Minn. A13	36	S−	Cream-tan	7	Abundant
Minn. A13 x Minn. F.1	36	S+	D	16	do
do 2	46	M	Cream-tan	1	do
do 3	37	S+	D	2	do
do 4	50	M	L	0	do
do 5	43	S	Lilac	0	do
do 6	34	S+	L	0	do
do 7	41	Sm	L	0	do
do 8	41	Sm	L	0	do
do 9	33	S+	L	4	do
do 10	36	S	L	9	do
do 11	40	S	L	20+	do
do 12	47	M	L	1	Moderate
do 13	50	M	L	0	do

* All segregates of different colors, except that 7 was similar to 8.

† S = sporidial; M = mycelial; Sm = semi-mycelial.

‡ D = darker than the lilac parent; L = lighter than the cream-tan parent.
+ and − indicate degrees of the type indicated.

Pathogenicity

The author is of the opinion that, while the proper complement of sex factors in the dikaryons is normally essential to enable *U. zeae* to parasitize the corn plant successfully, there are factors for pathogenicity in addition to those for sex. This fact became clearly evident in observing the degree of infection caused by the various combinations listed in Tables II, III, and IV. The various dikaryophytes that formed chlamydospores were by no means equally virulent. Some caused heavy infection, some light, and others very light infection.

The degree of infection is not recorded in Tables III and IV because the facts are shown clearly in subsequent tables.

The three lines that cause infection singly also appear to differ in virulence, altho all of them can produce chlamydospores. A13 x D.10 caused light or very light to incipient infection throughout, while line 6 caused heavy infection in most cases. In the discussion of Table IV evidence of differences in pathogenicity of different combinations of lines also is given. Additional experiments were made and the results recorded in Tables IX and X.

TABLE IX

RESULTS OF INOCULATING SIX SELFED LINES OF CORN IN THE GREENHOUSE WITH f_1 LINES FROM MINN. A13 x MINN. F AND WITH THE PARENTAL LINES IN COMBINATION WITH ONE ANOTHER

Selfed lines of corn	Minn. A13 x Minn. F	(Minn. A13 x Minn. F.1) x (Minn. A13 x Minn. F.9)	(Minn. A13 x Minn. F.6) x (Minn. A13 x Minn. F.9)
	Infection*	Infection	Infection
S-1	8/50 L	35/35 H	41/42 H
S-2	5/44 L	30/30 H	23/24 H
S-3	3/43 I	21/21 L	21/26 M
S-4	7/41 L	22/22 H	28/28 H
F-1	3/20 I	18/18 L	32/36 L
F-2	2/28 I	18/30 I	32/32 L

*The denominator denotes the number of plants inoculated; the numerator, the number infected.

H = heavy infection; M = moderate; L = light; I = incipient, i.e., galls very small and wart-like.

During the winter of 1928-29 a comparative pathogenicity test was made in the greenhouse with the following combinations: (Minn. A13 x Minn. F.1) x (Minn. A13 x Minn. F.9); (Minn. A13 x Minn. F.6) x (Minn. A13 x Minn. F.9), and the parental lines, Minn. A13 x Minn. F. Six selfed lines of corn were inoculated when about 8 to 12 inches high. The results are given in Table IX. Combinations of the two f_1 lines proved to be much more virulent than the parental combination. This may not be a fair comparison as far as the relative pathogenicity of the parental combination and that of the f_1 lines is concerned, because at the time the original cross, Minn. A13 x Minn. F, was made in the winter of 1928, the parental lines were strongly pathogenic, while subsequent tests proved that Minn. A13

had lost some of its original virulence. The results do show, however, that the pathogenicity of different combinations may be quite different.

A more extensive test on parasitism was made in the field. The combinations mentioned above were used. In addition, Minn. A13 x Minn. F.7 was back crossed with Minn. F; and Minn. A, the parent of Minn. A13, also, was crossed with Minn. F. Ten selfed lines of sweet corn were inoculated with the five combinations. The corn plants were 18 to 24 inches high and jointing at the time of inoculation. Duplicate plots were inoculated with each combination. The data are presented in Table X. There were decided differences in the virulence of the different combinations. In descending order of virulence, they rank as follows: (Minn. A13 x Minn. F.6) x (Minn. A13 x Minn. F.9); (Minn. A13 x Minn. F.1) x (Minn. A13 x Minn. F.9); (Minn. A13 x Minn. F.7) x Minn. F. The other two combinations were relatively weak in virulence. The results were consistent in both series.

TABLE X

Results of Inoculating Ten Selfed Lines of Corn in the Field with f_1 Lines from Minn. A13 x Minn. F, in Combination with One Another or with Minn. F, and with Three Other Monosporidial Lines in Combination with One Another

Selfed lines of corn	(Minn. A13xMinn. F.1)x (Minn. A13xMinn. F.9)	(Minn. A13xMinn. F.6)x (Minn. A13xMinn. F.9)	Minn. F x (Minn. A13xMinn. F.7)
	Infection*	Infection	Infection
S-4	21/32 L to M ±	33/40 L to H +	27/37 L to M +
S-5	30/32 I to M ±	28/29 L to H ++	14/24 I to M +
S-6	24/27 L to H ±	28/30 L to H ++	17/30 I to L ±
S-7	11/17 I to L ±	21/21 H +++	11/16 I to L ±
S-8	19/32 L to M ±	29/29 M to H ++	19/30 I to L ±
S-9	7/24 L to M ±	22/23 L to H +++	11/21 L to M +
S-10	5/31 I to L ±	25/32 I to H +	12/30 I to L ±
S-11	16/28 I to L ±	31/33 M to H ++	17/29 I to L ±
S-14	10/19 L to M ±	23/23 L to H ++	5/16 I ±
S-15	32/33 L to H ±	36/36 L +++	18/23 ± to M +

TABLE X—*Continued*

Selfed-lines of corn	Minn. A13 x Minn. F	Minn. A x Minn. F	Checks†
	Infection	Infection	Infection
S-4	3/25 I to L ±	3/25 L ±	1/22 L +
S-5	6/17 I ±	6/25 I to L ±	0/16
S-6	5/26 I to L ±	5/17 I to L ±	0/17
S-7	2/14 I to L ±	5/20 I to L ±	0/12
S-8	8/34 I to L ±	8/26 I to L ±	1/18 L ±
S-9	9/22 L ±	5/21 L to M ±	0/16
S-10	3/29 L ±	2/25 I to L ±	0/19
S-11	4/26 I to L ±	4/29 I to L ±	0/20
S-14	5/21 I to L ±	2/20 I ±	0/21
S-15	11/29 I to L ±	12/28 I to L ±	0/21

* The denominator denotes the number of plants inoculated; the numerator, the number infected. Plus and minus signs indicate the number of galls; +++ indicate very many galls; — indicates few galls.

H=heavy infection; M=moderate; L=light; I=incipient, i.e., galls very small and wart-like.

† Inoculated with Minn. A13 x Minn. F.6.

It appears certain from all of the observations and experiments that there are multiple factors for pathogenicity and that the virulence of the pathogene in the field depends on the chance combinations of gametic lines with the proper complement of factors for pathogenicity and sex.

CONCLUSIONS

The results of experiments with combinations of monosporidial lines substantiate the previous conclusions of Christensen and Stakman (2) that there are parasitic races or physiologic forms of *U. zeae*, and that selfed lines of corn may be physiologically resistant to some forms and susceptible to others. The degree of infection depends upon the lines of smut and the lines of corn involved. It is clear that the pathogenicity of combinations of lines probably is the component of the

factors for pathogenicity, and to a certain extent of those for sex, of the smut lines causing infection. A monosporidial line of *U. zeae* may be strongly pathogenic in combination with certain lines of opposite sex and only weakly pathogenic with other lines of opposite sex. It seems clear that different lines have different factors for pathogenicity, apart from those for sex. The most conclusive evidence, however, that monosporidial lines differ with respect to pathogenicity as such, is the fact that those which can cause infection alone also have different degrees of virulence.

While it was shown that some monosporidial lines, alone, can cause infection, it seems evident that *U. zeae* is predominantly heterothallic. As there evidently are multiple factors for sex and pathogenicity, there may be a great many different combinations of sexual and pathogenic potentialities. While the ultimate implication of these facts depends upon the number of factors in existence, it is significant that so many different combinations are possible. *U. zeae* is very heterozygous for most characters. This means that great variation is possible and that there may be a large number of combinations resulting in the production of a great many different lines.

There is still further evidence from the results recorded here that haploid lines are clones and that the so-called mutations are due to genotypic changes. The corn smut pathogene, therefore, comprises many lines now, and new ones continually are arising by mutation and hybridization. It seems likely that attempts should be made to produce lines of corn with functional or structural resistance. It is possible that some lines of corn may have physiologic resistance to all of the combinations of physiologic forms with which they are likely to be inoculated under natural conditions. However, it seems probable that lines of corn with functional or structural resistance will be most useful. There is some evidence that such lines are in existence, but information regarding the mode of infection and the nature of resistance still is very meager. Therefore, much more investigation is necessary before the practical implications of the results obtained can be known.

SUMMARY

1. Monosporidial lines from Canada, France, Hungary, Italy, Spain, and from several places in the United States produced smut galls when paired with two monosporidial lines of opposite sex isolated from chlamydospores from Minnesota. There was no evidence, however, of complete interfertility or intersterility between possible "Geographic strains."

2. Chlamydospores were obtained from six different crosses of

monosporidial lines, including some mutants. One hundred and two sporidia (F_1 gametes) from these crosses were isolated and the resulting monosporidial lines (designated as f_1 lines) were studied in considerable detail.

3. A study of these f_1 lines on potato-dextrose agar indicates that there are multiple factors in *U. zeae* for rate of growth; for type of growth; for color, consistency, and topography of colonies; and, possibly, for the tendency to mutate. Thirty-two f_1 lines isolated from chlamydospores in a single smut gall resulting from crossing two monosporidial lines were all culturally different from each other.

4. There apparently are multiple factors for sex and pathogenicity.

5. Dikaryophytes resulting from pairing f_1 lines from a single cross may have various degrees of pathogenicity: some are very virulent, some moderately virulent, and others appear to be relatively innocuous.

6. *U. zeae* is predominantly heterothallic but three monosporidial lines obtained by isolating F_1 gametes from a cross cause normal infection when inoculated singly into corn plants, although they have different degrees of virulence. Thirty-one other lines from the same cross did not cause infection when inoculated singly into corn.

7. A study of the growth type of f_1 lines isolated from crosses between certain mutants and their parents and between the mutants and another monosporidial line indicates rather clearly that the so-called mutants are true mutants resulting from genotypic changes.

8. It will be necessary to learn more about the mode of infection, the nature of resistance of lines of corn to smut, and the performance of these lines in the field before the practical importance of the facts presented in this paper can be known.

LITERATURE CITED

1. Boss, Georg. Beiträge zur Zytologie der Ustilagineen. Planta **3**:597-627. 1927.

2. Christensen, J. J., and Stakman, E. C. Physiologic specialization and mutation in *Ustilago zeae*. Phytopath. **16**::979-999. 1916.

3. Eddins, A. H. Pathogenicity of multisporidial and monosporidial cultures of *Ustilago zeae* (Beckm.) Ung. (Abstr.). Phytopath. **19**:91. 1929.

4. Goldschmidt, Viktor. Vererbungsversuche mit den biologischen Arten des Antherenbrandes (*Ustilago violacea* Pers.). Ein Beitrag zur Frage der parasitären Spezialisierung. Ztschr. Bot. **21**::1-90. 1928.

5. Hanna, W. F. The problem of sex in *Coprinus lagopus*. Ann. Bot. **39**:431-457. 1925.

6. ———. Studies in the physiology and cytology of *Ustilago zeae* and *Sorosporium reilianum*. Phytopath. **19**:415-442. 1929.

7. Kniep, H. Über erbliche Änderungen von Geschlechtsfaktoren bei Pilzen. Ztschr. Induktive Abstammungs- und Vererbungslehre **31**:170-183. 1923.
8. ———. Die Sexualität der niederen Pflanzen. Jena, 1928.
9. ———. Vererbungserscheinungen bei Pilzen. Biblio. Gen. **5**:372-478. 1929.
10. Stakman, E. C., and Christensen, J. J. Heterothallism in *Ustilago zeae*. Phytopath. **17**:827-834. 1927.
11. Vandendries, R. Nouvelles recherches sur la sexualité des Basidiomycètes. Bull. Soc. Roy. Bot. Belgique **16**. 1923.
12. ———. Nouvelles recherches expérimentales sur le comportement sexuel de *Coprinus micaceus*. Mémoires l'Acad. Roy. Belgique **9**:1-128. 1927.

PLATES

PLATE I

Golden Bantam Corn Inoculated with Monosporidial Lines of *Ustilago zeae*.

On the left, Minn. A13 x Minn. D.6.1. On the right, Minn. A13 x Minn. D.6.2.3. Both lines caused normal infection when inoculated singly into the corn plants. So far only three lines have been found that can cause infection singly.

PLATE I

PLATE II

Eight monosporidial f_1 lines from cross, Minn. A13 x Minn. D, on potato-dextrose agar, showing differences in cultural characteristics.

PLATE II

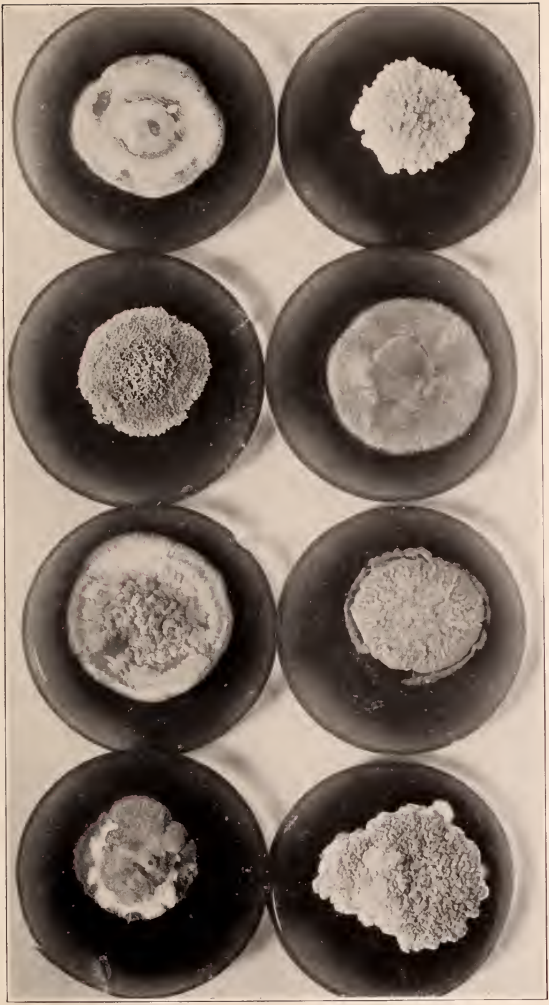


PLATE III

Eight monosporidial f_1 lines from cross, Minn. A12 x Minn. D, on potato-dextrose agar, showing differences in cultural characteristics.

PLATE III